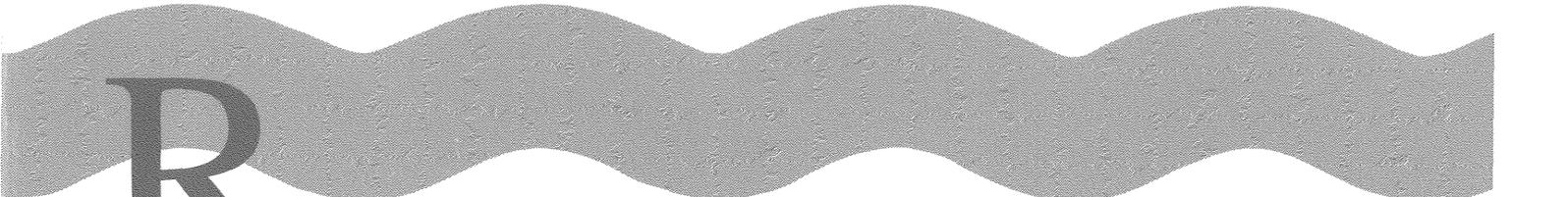




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Effect of Different Parameters on Settling Properties  
of Activated Sludge

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# Summary

This literature review is a summary of process factors affecting the settling properties of activated sludge. The separation of biomass from the purified water is a critical part of the activated sludge process. The settlers have three functions; to produce a supernatant containing enough low concentrations of suspended solids to satisfy given effluent standards, to produce settled sludge which is enough thickened to maintain a desired concentration of activated sludge in the aeration tank and to act as a storage tank for sludge at high hydraulic loadings. The thickening process is favoured by large, regularly shaped and compact flocs, while the clarification process is favoured by more irregularly shaped flocs which sweep smaller flocs with them during settling.

Sometimes the settling process works inefficiently due to settling problems such as: pin-point flocs (very small flocs), bulking sludge (caused by excessive growth of filamentous and/or Zoogloea bacteria), rising sludge ( $N_2$ -bubbles formed due to denitrification stick to flocs and lift them to the surface) and foaming. Activated sludge flocs are then carried out with the effluent. This increases the concentration of BOD as well as phosphorus (phosphorus is generally particle bound) in the effluent. It can, in severe cases, lead to flooding of the settlers and the whole process is disturbed due to a decrease in sludge age.

The factors affecting the settling process can be divided into physical and biological/chemical factors which interact in a complex way. The process parameters which most affect the settling properties are sludge age, hydraulic regime in the aeration tank, mixed liquor suspended solids concentration, degree of turbulence and dissolved oxygen concentration. High concentrations of suspended solids can also be caused by a too high surface or suspended solids load on the settler or inappropriate flow patterns which can scour the sludge blanket. The bioflocculation process is not fully understood but there are a few suggested mechanisms; the bacteria excrete exocellular polymers which joins the cells together by means of divalent cations; the cells are negatively charged and they are joined together by cations; some bacteria form long filaments onto which other bacteria can adhere and that the interaction between the floc components can be described by the DLVO-theory. It is also believed that hydrophobic and hydrophilic surface properties play an important role.

The composition of the wastewater can affect the size and structure of the activated sludge flocs. High sulphide concentration, lack of certain nutrients like nitrogen and phosphorus, high carbohydrate concentration and high concentration of lipids are believed to enhance growth of filamentous bacteria which can cause bulking sludge. Contaminants in the wastewater can also affect the bioflocculation process.



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# 1. Introduction

In wastewater treatment, the quality of the effluent is to a large degree dependent on how well the activated sludge can be separated from the treated water. The separation is generally done by settling of spontaneously formed flocs of activated sludge. This process is associated with many problems such as formation of flocs with poor settling properties and floating sludge which can cause loss of sludge from the settler into the effluent. The efficiency of the settling process is dependent on both physical (e.g. design and operation of the settlers, turbulence in the aeration tanks) and chemical/biological factors (e.g. degree of bioflocculation, wastewater characteristics and composition of the microflora) and this makes the process very complex. The settler and the aeration tank have to be seen as an interacting system. Although a considerable amount of research has been done within this field, the factors affecting the settling properties of activated sludge are not fully understood. This is seen by the interdependency of many variable factors.

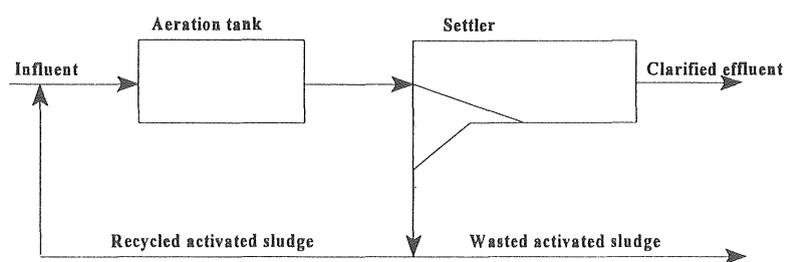
As wastewater plants are rebuilt for nitrogen removal, the settlers will be subject to higher loadings due to the high concentrations of suspended solids required to minimize the aeration tank volumes (nitrifying bacteria have a low growth rate). An expansion of the settlers is not always possible due to limited space available as well as limited funds and it is therefore necessary that the existing settlers work efficiently. Thus it is important to know which factors affect the settling process of activated sludge to be able to operate the wastewater plant in such a way that a sludge with good settling properties is produced.

This literature review is a summary of the factors, both physical and biological, known to affect the formation of activated sludge flocs and the principles behind the settling process. The areas of settler design and settling principles are treated in brief. The literature summarized is intended to be the base for experimental work to further study which parameters affect the settling properties of activated sludge.

## 2. The Activated Sludge Process

In biological treatment of wastewater, dissolved and colloidal organic material are degraded by means of microorganisms which spontaneously form flocs. The major objectives are to reduce the amount of biologically degradable organic material and, in some cases, to remove nutrients like nitrogen and phosphorus. One part of the organic material in the wastewater is decomposed and one part is transformed into new cell material. For the synthesis of cell material, compounds such as C, N, P, H, O, and S must be present in certain amounts. Carbon is usually the growth limiting compound in domestic wastewater while industrial wastewaters are normally nutrient deficient (for example nitrogen and phosphorus). The biological process can be combined with chemical treatment for precipitation of organic material and nutrients (especially phosphorus).

The activated sludge process was developed in England by Arden and Lockett in 1914 and the process has been subjected to many improvements throughout the years (Metcalf and Eddy, 1979). Basically, the conventional activated sludge process consists of an aerated suspension of a mixed bacterial culture which carry out the biological conversion of the contaminants in the wastewater. The suspension is supplied with oxygen by means of compressed air, pure oxygen or by mechanical aeration. After a certain contact time between the wastewater and microorganisms, the suspension is transferred to a settler where the microorganisms are separated gravimetrically from the wastewater to produce a clear effluent. One part of the settled biomass is recycled back to the inlet of the aeration tank and one part is wasted as excess sludge. The recycling ensures a continuous sludge inoculation and extends the sludge residence time to give the microorganisms a chance to adapt to the new environment and enables the adsorbed organic material to be oxidized. The recirculation also reduces the aeration tank volume. The amount of wasted sludge is the same as the sludge production to avoid accumulation of biomass in the system. Sludge production is dependent on the type of process used, the operation of the wastewater plant and on the wastewater characteristics. A schematic drawing of the activated sludge process is presented in Fig. 2.1.



**Figure 2.1.** Schematic drawing of a conventional activated sludge plant.

Many modifications to the activated sludge process have been made to facilitate the remove of nutrients like nitrogen and phosphorus (Metcalf and Eddy, 1979).

A special property of activated sludge is its high adsorption capacity of suspended material. Some organic molecules are oxidized immediately while others are more slowly oxidized. The initial step in removing suspended material from wastewater is attachment onto the flocs. This process is dependent on the adsorptive capacities of the flocs. The following step is oxidation of the adsorbed particles (enzymes break down the larger particles into smaller constituents which can pass through the cell wall) and the degree of oxidation is improved with contact time. A modification of the conventional activated sludge process is contact stabilization where the recycled sludge is subjected to aeration before entering the aeration basin. Attached organic material which was not removed in the aeration basin is then degraded to recover the adsorption capacity of the activated sludge.

### 3. Separation of Activated Sludge Flocs

In the activated sludge process, separation of cell mass and liquid medium normally occurs via bioflocculation and gravity settling. The effectiveness of the separation process is dependent on the degree of bioflocculation, the physical characteristics of the activated sludge flocs and on the design and operation of the settling tank. Very small flocs have a density near that of water and cannot be separated by settling. A subsequent step using filtration or flotation can be used to polish the effluent from non-settlable flocs. Chemicals such as aluminium sulphate and synthetic polymers can be added to improve flocculation of activated sludge. If aluminium sulphate is added, aluminium hydroxide is precipitated onto which colloidal particles can adsorb. Synthetic polymers create bridges between particles (the polymer has a chemical group which can react with the surface of the particle) which form larger flocs. There are three types of synthetic polymers; anionic, cationic and nonionic and they are added under high turbulence in the transport channel between aeration tank and settler.

After separation, the organic material in the effluent is composed of the following constituents (Metcalf and Eddy, 1979):

- 1) soluble biodegradable organics (organics that passed through the biological treatment, intermediate products from the microorganism's metabolism and cellular components);
- 2) suspended organic material (activated sludge flocs that were not separated in the settling tank and colloidal material entering the treatment plant with the wastewater and that was not removed in the biological treatment);
- 3) non-biodegradable, inert organics (present in the incoming wastewater and by-products of biological degradation).

#### 3.1. Settling

The settling process occurring in the settler is a critical part of the activated sludge process. It is often the last treatment step before the effluent is transported to the recipient. Its performance is measured by the quality of the effluent and the quality of the thickened sludge. The thickened sludge must be thick enough to be able to recirculate it to the aeration tank to maintain a desired concentration of biomass. Wasted sludge withdrawn from the system must also be easy to handle and to treat. The suspended solids which escape the separation in the settling tank contribute to most of the BOD in the effluent. Another important factor which makes it desirable to remove as much suspended solids as possible is the content of particulate bound phosphorus in the activated sludge flocs.

Heavy metals, toxic organic compounds and pathogenic microorganisms can also be discharged to the effluent with activated sludge flocs.

Flocculation is the process where microorganisms and suspended or colloidal components form larger aggregates and it is decisive for the removal efficiency of the secondary settler. For an efficient separation, two processes must operate satisfactory: the sludge must be thickened enough to maintain a desired concentration of activated sludge in the aeration tank and the supernatant must contain low enough concentrations of suspended solids to satisfy given standards. If these processes fail, the immediate result is an increase of suspended solids in the effluent and the activated sludge process could deteriorate due to a too low sludge age. The secondary settler serves also a function of storage tank for activated sludge during periods of higher hydraulic load. Therefore, when designing a secondary settler an extra volume should be added to cope with fluctuations in the inflow.

There are several types of settling problems. Some of them are directly related to the structure of the flocs (morphology) and some are related to substances coming in with the raw wastewater or which are produced during the metabolism.

***pin-point flocs:*** The problem that has the greatest impact on effluent quality is poor clarification, ie small flocs (pin-point flocs) or dispersed bacteria which do not form larger or attached flocs and, consequently, cannot be separated by gravity settling (their density is near that of water). Poor clarification which gives rise to a turbid effluent is caused by inefficient flocculation or the break-up of flocs due to shear forces.

***filamentous microorganisms:*** The excessive growth of filamentous bacteria (Eikelboom and van Buijsen, 1981) causes settling problems in that it produces a sludge with less efficient compaction ability (bulking sludge). Most activated sludges contain filamentous microorganisms and they can contribute to a more clear effluent in that small flocs can adhere to the network of filaments during settling. A certain number of filaments can contribute to stronger flocs, but problems occur when filamentous microorganisms grow in excessive numbers. There are many types of filamentous bacteria which can thrive in different environments and a few of them have been classified (Eikelboom and van Buijsen, 1981).

***Zoogloea bulking:*** The Zoogloea microorganisms form finger-like colonies and they excrete exocellular slime which gives the flocs a very voluminous character. It can in extreme cases lead to foaming and scum formation (Novák et al., 1993, 1994). This causes increased concentrations of suspended solids in the effluent. The Zoogloea growth can be related to the composition of the wastewater and lack of certain nutrients such as N, P, Ca, Mg and Fe.

***rising sludge:*** High concentrations of suspended solids in the effluent can also be caused by rising sludge. As a result of biological denitrification nitrogen gas is formed. The nitrogen gas is poorly soluble and bubbles can adhere to activated sludge flocs and float them to the surface of the settler (Henze et al, 1993).

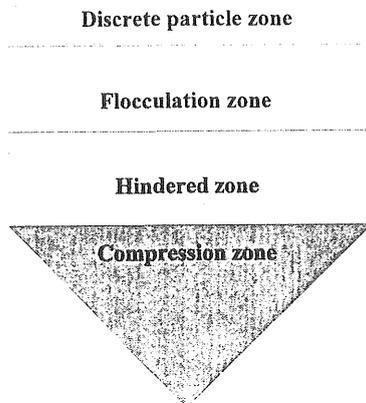
*scumming*: Another common problem is the formation of foam caused by non-degradable surfactants or by the presence of *Nocardia* sp., *Actinomyces* or *Microthrix parvicella* (types of filamentous bacteria). The sludge is then transported to the surface and it can overflow the settler. A review over scumming and bulking problems is given by Kappeler (1994). Blackall (1994) has made a molecular identification of activated sludge foaming bacteria.

### 3.2. Settling: basic principles

Settling is the separation of suspended particles which are denser than water by means of gravity. Larger particulate material is generally removed in a primary settler ahead of the aeration basin and biological flocs and chemical flocs (if a chemical coagulation process is used) are removed from the treated water in a secondary settler. The purpose of the settler is dual: to produce a clarified effluent and to produce a sludge with a high solids concentration which enables recirculation of sludge to the aeration tank to maintain a desired concentration of biomass. Thus, two main processes are taking place simultaneously in a sedimentation tank:

- Clarification
- Thickening

Depending on the degree of interaction between particles, the settling process can be divided into four zones: 1) discrete particle zone 2) flocculation zone 3) hindered zone and 4) compression zone. This is illustrated in Figure 3.1.



**Figure 3.1.** The four settling zones.

In the discrete particle zone, the particles settle independently of each other (also called clarification). For a spherical, discrete particle in the laminar flow regime, the particle settles with a velocity,  $v_s$ , according to Stokes' equation:

$$v_s = \frac{g}{18}(\rho_p - \rho_v) \frac{d_p^2}{\eta}$$

where

$v_s$  = settling velocity [m/s]

$g$  = gravity coefficient [ $m/s^2$ ]

$\rho$  = density of particle resp. liquid [ $kg/m^3$ ]

$d_p$  = particle diameter [m]

$\eta$  = dynamic viscosity of the liquid [ $kg/m \cdot s$ ]

As can be seen from this, the settling velocity is mainly dependent on the particle size and density.

In reality, activated sludge flocs are not spherical but more irregularly shaped. This creates a higher hydraulic resistance and they settle slower (Newton's law):

$$v_s = \sqrt{\frac{2g(\rho_p - \rho_v)V_p}{C_D\rho_v A_p}}$$

where

$v_s$  = settling velocity [m/s]

$C_D$  = Newton's resistance coefficient

(dependent on particle shape and Reynold's number) [-]

$V_p$  = particle volume [ $m^3$ ]

$A_p$  = surface area vertical to the flow [ $m^2$ ]

Hazen (1904) developed an ideal horizontal flow model to describe the clarification process. It is valid for discrete particles. In principle, it means that all particles with a settling velocity larger than  $v_f$  will settle while passing an ideal settler from one end to the other (assuming that all particles move with identical velocity vectors).

$$v_f = \frac{Q}{A}$$

where

$v_f$  = settling velocity [m/s]

$Q$  = flow [ $m^3/s$ ]

$A$  = sedimentation area [ $m^2$ ]

The ratio  $Q/A$  is often called the overflow rate and is the capacity limit for the process (for an idealized settler). For a continuous settler, the depth of the settler and the detention time should be such that:

$$v_f = \frac{\text{depth}}{\text{detention-time}}$$

where

$v_f$  = settling velocity [m/s]

When the capacity limit is exceeded, only a fraction of the flocs will be separated; ie the particles in the lower region of the influent stream (assuming that particles of various sizes are uniformly distributed over the entire depth of the basin at the inlet). This theory requires that the flow is uniformly distributed over the whole cross-section area of the sedimentation tank. Camp (1936) made a modification of Hazen's theory to take into account settling velocity distribution. He suggested that particles with a settling velocity less than  $v_f$  would be removed with a fraction  $f$  (Figure 3.2):

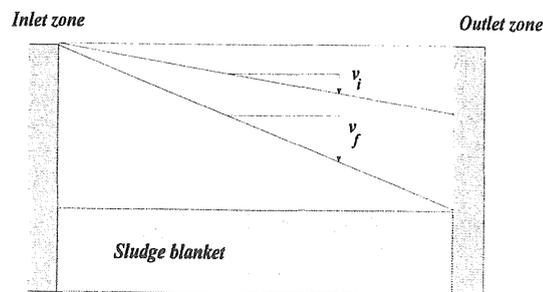
$$f_i = \frac{v_i}{v_f}$$

where

$f_i$  = removal of fraction  $i$  [-]

$v_i$  = settling velocity of fraction  $i$  [m/s]

$v_f$  = overflow rate [m/s]



**Figure 3.2.** Schematic drawing of an ideal settler.

In reality vertical velocity gradients exist and the capacity for the settler is lower than calculated. This is generally accounted for by over-design. The above theories also do not take into account that smaller flocs adhere to each other during the sedimentation process and the presence of a density current. The density current is formed due to differences in density between the mixed

liquor entering the basin and the bulk concentration of the liquid in the basin and most of the particles probably settle out there.

Fitch (1979) proposed that for a flocculent suspension clarification is a two-step process: first the particles flocculate and then the flocculates settle out. If the flocculation is rapid (or the suspension is pre-flocculated before entering the settler), i.e. the settling velocity is constant over the entire sedimentation time, the surface area of the settler will govern the removal of solids. If the flocculation is slow, the removal will be governed by the flocculation kinetics and thus the retention time (and thereby dependent on the tank depth).

As the concentration of particles increases they start to interact with each other and they coalesce to form larger aggregates which, due to the increased mass, settle faster. When the concentration of particles reaches a certain critical level, the interparticle forces are so strong that the particles are fixed in position relative to each other and the whole mass of particles settles as a unit and an interface develops between the settling particles and the liquid. In the compression zone the particles begin to feel the pressure from the layers of particles above and a further settling can only occur as a consequence of compression. This is the opposite to settling in that the liquid is transported away from the particles. In a settler, clarification occurs in the upper part and thickening in the lower part.

Settlers can be designed according to the solids flux theory (Coe and Clevenger, 1916, Dick, 1970). The composition of different wastewaters varies considerably and settling tests are generally required to determine the settling characteristics. Ideally, when the settler is operated at steady state, a constant flux of solids is transported downwards. The transport is due to two mechanisms: 1) flow due to gravity and 2) flow due to the underflow of return sludge. The gravity component can be written as:

$$M_g = C_i V_i$$

where

$M_g$  = solids flux due to gravity [kg/m<sup>2</sup>h]

$C_i$  = conc. of solids at point i [g/m<sup>3</sup>]

$V_i$  = settling velocity at point i [m/h]

The under flow component can be written as:

$$M_u = C_i \frac{Q_u}{A}$$

where

$M_u$  = mass flux due to the bulk movement [kg/m<sup>2</sup>h]

$C_i$  = conc. of solids at point i [g/m<sup>3</sup>]

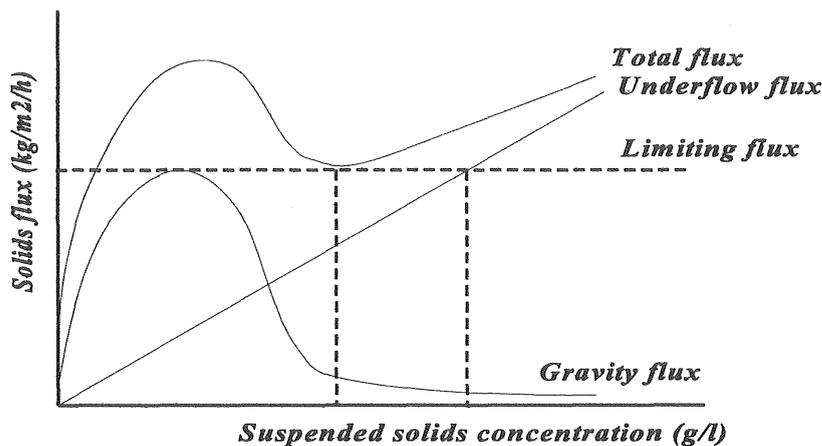
$A$  = cross-section area [m<sup>2</sup>]

$Q_u$  = under flow rate [m<sup>3</sup>/h]

The total flux of solids,  $M_t$ , is then:

$$M_t = M_g + M_u$$

The solids flux due to gravity can be calculated by performing settling velocity tests at different initial concentrations (the initial settling velocity is measured as the steepest slope of the settling curve). The solids flux due to the underflow is a linear function of solids concentration. If the two solids flux curves are added, a local minimum can be seen. This is the limiting flux. If more solids are fed to the basin than the limiting solids flux, the solids will eventually build up in the settler. A schematic drawing of a solids flux curve is found in Figure 3.3 (a more detailed analyse of the solids flux method can be found by Metcalf and Eddy, 1979).



**Figure 3.3.** Schematic drawing of a solids flux curve.

To be able to use the solids flux theory for the design of secondary settlers, the settling velocity has to be determined. A number of models (empirical or theoretical) which correlate the suspended solids concentration to the settling velocity have been developed. Cho et al (1993) have made a summary of different models and also compared six different models. The settling properties of activated sludge changes continuously, and therefore it is desirable to find rapid and easy methods which are able to measure it on-line.

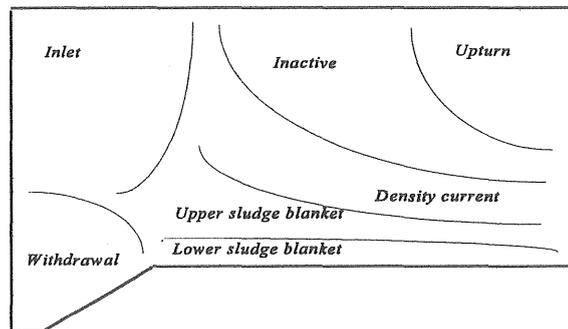
There are many 'rules of thumb' in the design of final settlers (Dick, 1976) and they are based on empirical parameters. In the last years a more 'scientific' approach has been put forward. The most common design parameters have been hydraulic loading rate, hydraulic retention time, side wall depth and outlet weirs. The design of settlers were often in the past based on the steady-state theories of Hazen (1904) and Camp (1936). This is, however, an over-simplification and the settlers normally operate under dynamic conditions. This is normally compensated for by over-

design. Only a brief summary of the design criteria is given here.

The surface load is the most common design parameter. If the flow in the settler is considered as being vertical, the flow must not exceed that of the settling velocity of the slowest particles (Hazen, 1904). A high hydraulic loading rate can also create turbulence in the settler, which can affect the sludge blanket (the increased flow in the density current can scour the sludge blanket), and cause higher concentrations of suspended solids in the effluent.

The importance of side wall depth has been subjected to much research. According to the classical settling theory by Hazen (1904), the settling performance is independent of depth. Later studies have shown that the depth has impact on the effluent quality. If the settler is too shallow the over flow stream can scour the sludge blanket. In a deeper tank, the sludge blanket level is further below the effluent weirs and the risk for sludge to escape over the weir is reduced (Pflanz, 1969); i.e. the settler has a buffer effect.

Lumley and Balmér (1990) studied the solids transport in a rectangular settler, and they distinguished seven transport zones. It was found that the transport mechanism in the sludge blanket is predominantly horizontal near the outlet end and becomes increasingly vertical as the inlet end is neared (see Figure 3.4). Most of the removal of sludge took place within the first two thirds of the settler and the turnover rate of the sludge in the outlet end of the sludge blanket was low.



**Figure 3.4.** Transport zones in a secondary settler (Lumley and Balmér, 1990).

The oxygen concentration drops in the lower part of the settler. For a plant with enhanced nutrients removal, denitrification can start if the retention time is too long. This can cause flotation of sludge flocs (due to formation of  $N_2$  bubbles), release of phosphorus and filamentous growth. Audic et al (1994) injected a tracer (radioactive gold) to two full-scale settlers; a circular scraper-type and a radial suction tube type. For the circular settler, 10 to 30% of the sludge short-circuited. About 40 to 50% of the sludge remained in the system for more than 5 hours. For the suction settler, a perfect sludge distribution with time was observed for a thin sludge blanket (90% of the sludge was recycled within 2 hours) but for a thick sludge blanket 60% of the sludge remained longer than 5 hours.

As a summary, it can be said that to avoid lost of suspended solids to the effluent three criteria must be satisfied:

- 1) The thickening capacity of the secondary sedimentation tank must not be exceeded, i.e. the load of suspended solids must not exceed the solids flux.
- 2) The clarification capacity of the secondary sedimentation tank must not be exceeded, i.e. the overflow rate must not be higher than the settling velocity of the activated sludge flocs.
- 3) Account for varying sludge characteristics.

There are many different types of settlers; both circular and rectangular. The circular ones are normally 10-30 m in diameter and center-fed or rim-fed. Rectangular settlers have usually a length-to-width ratio of 6 or more to give a good flow pattern. However, the settling principles are the same for both round and rectangular tanks. The settled sludge is removed from the bottom of the tank by scrapes. There are several literature reviews concerning design of secondary settlers (Parker, 1983, Dick, 1976, Lumley, 1985, Metcalf & Eddy, 1979).

## 4. Wastewater Characteristics

The composition of wastewater depends on its source and there are two main types; industrial and domestic. Often, industrial and domestic wastewaters are mixed and treated in the same wastewater plant (the industrial wastewater is often pretreated before discharge to the sewer system). This can cause large problems since toxic compounds in industrial wastewater can damage the culture of microorganisms in the treatment plant.

Wastewater contains a variety of microorganisms and they provide a continuous inoculation of mixed-culture for the treatment process. The strength of wastewater is normally measured in terms of biochemical oxygen demand (BOD) which is equal to the amount of dissolved oxygen which is consumed by activated sludge incubated for a certain time at 20°C. Another indicator of wastewater strength is the chemical oxygen demand (COD). It is equal to the number of milligrams of oxygen which a litre of sample will adsorb from a hot and acidic solution of potassium dichromate. This value is normally higher than BOD but it has the advantage of being analysed within 2 hours. Both BOD and COD gives a coarse estimation of the wastewater composition but they are valuable tools in getting information about the performance of the process. The main organic constituents in wastewater are protein, carbohydrate and lipid (Nielsen et al. 1992). The concentration of suspended solids, nitrogen and phosphorus are also important parameters. Heavy metals and toxic organic compounds can be present in considerable concentrations.

COD can be divided into three major categories according to the rate of biodegradation (Wanner, 1994):

***Readily biodegradable compounds:*** Organic compounds with low molecular weight which can be immediately metabolized by the bacterial cells (methanol, ethanol, volatile fatty acids, monosaccharides and lower amino acids).

***Slowly biodegradable compounds:*** Compounds which have to be hydrolyzed by means of extracellular enzymes before they can be transported into the cells (soluble or suspended).

***Unbiodegradable compounds:*** Compounds which pass through the biological treatment and remain in the effluent. They can also be generated during the biological treatment (metabolism by-products).

Torrijos et al. (1994) characterized wastewater by means of sequencing batch reactors. The wastewater was filtrated into three fractions; a soluble fraction ( $< 0.1 \mu\text{m}$ ), a colloidal fraction ( $0.1 \mu\text{m} << 50 \mu\text{m}$ ) and a particulate fraction ( $> 50 \mu\text{m}$ ). The soluble fraction was divided into a non-biodegradable fraction and a biodegradable fraction. A part of the soluble biodegradable fraction disappeared with a rate higher than the rate of oxygen consumption which indicates that they were adsorbed onto the activated sludge flocs.

The colloidal fraction was eliminated very fast due to adsorption onto the flocs and the biological degradation started a few hours later.

Nutrients like nitrogen and phosphorus are necessary for biological growth and the lack of those compounds can govern the growth of undesirable filamentous microorganisms. Too high concentrations of nitrogen and phosphorus in the effluent can lead to eutrophication of receiving waters. Due to microbial activity in the sewer system, most of the nitrogen entering the wastewater plant is in the form of ammonia and organic nitrogen. Phosphorus is present in the wastewater in inorganic (ortho- and polyphosphates) and organic forms. Enzymatic reactions convert most of the organic phosphorus into orthophosphate.

The contaminants in wastewater is a mixture of particulate and soluble compounds of different sizes. Factors such as settling rate, mass transfer, adsorption, diffusion and biochemical reaction rates are all dependent on particle size. Levine et al. (1985) discuss the importance of characterization of the size distribution of the contaminants in wastewater for developing a more fundamental understanding of the complex interactions in a wastewater plant. Small organic compounds can be taken up directly by the bacteria, while larger ones must first be broken down into smaller constituents by means of enzymes. Therefore, the size of the organic material in wastewater can affect the biological treatment and thereby the characteristics of the sludge flocs. The sizes of the most important constituents in wastewater are illustrated in Figure 4.1.

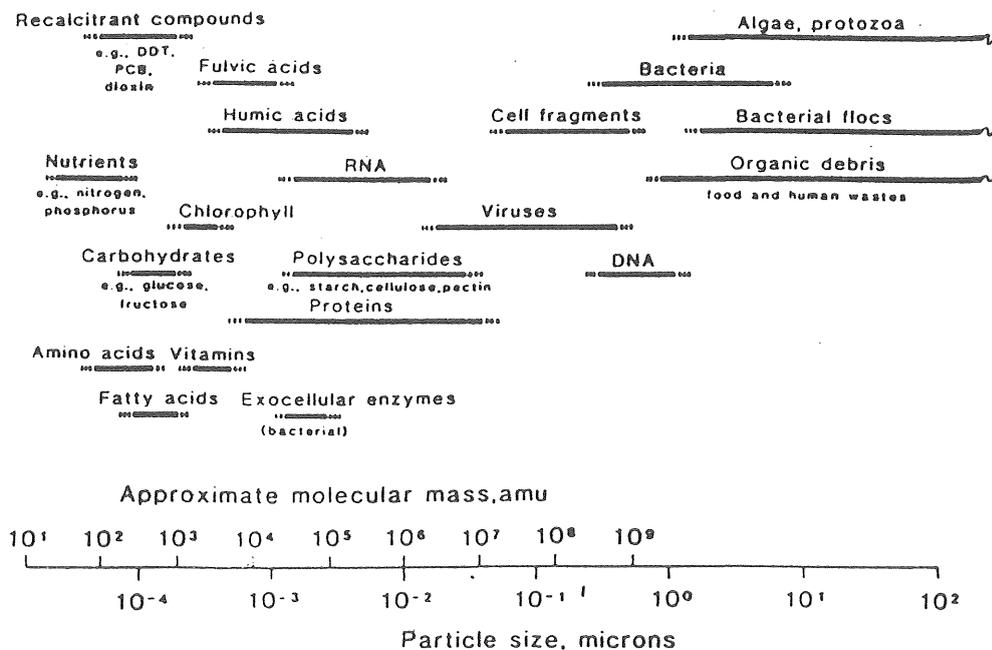


Figure 4.1. Typical organic constituents in settled municipal wastewater (from Levine et al, 1985).

Contaminants in wastewater are in general in the size range 0.001-100  $\mu\text{m}$  (Levine et al, 1985) and in the supernatant after settling in the size range 0.1-50  $\mu\text{m}$ . In their study it was found that 30 to 85 % of the organic material was larger than 0.1  $\mu\text{m}$  (the percentage depends, of course, on the sewer system and the community connected to it, etc).

Boller (1993) compared the size distributions in the different steps during the treatment of wastewater. In the aeration basin the particle size ranged from 1 to 1000  $\mu\text{m}$ , whereas after settling (ie in the supernatant) the maximum diameters were less than 150  $\mu\text{m}$ . The composition of raw wastewater could be fractionated accordingly: 45-55% dissolved components (< 0.001  $\mu\text{m}$ ), 20-35% colloidal particulates (0.001-1  $\mu\text{m}$ ) and 25-35% supra-colloidal particulates (1-100  $\mu\text{m}$ ). For settled as well as for unsettled wastewater, the colloidal particles < 10  $\mu\text{m}$  dominate the mass distribution in raw wastewater.

High concentrations of compounds like lipids and oils can negatively affect the settling properties of activated sludge (Forster, 1992). Few data that relate the composition of the wastewater to the settling characteristics of the activated sludge can be found in the literature. More knowledge about this is required to be able to predict the impact of for example new supposedly 'environmentally friendly' chemicals (containing various types of surface active compounds) on the floc structure and settling properties of activated sludge in wastewater plants.

## 5. Composition of Activated Sludge

Activated sludge is a complex mixture of different microorganisms (mainly different types of bacteria), dead cells and particulate organic and inorganic material. For the conventional activated sludge process, the population of microorganisms present depends on the composition of the wastewater and on the loading of the plant. There is a competition between different microorganisms for the available nutrients. Which microorganisms that are dominating is mainly decided by the efficiency in adaptation to the environmental conditions surrounding them. Bacteria are the most important microorganism for the degradation of organic material in wastewater and they are predominant in terms of biomass and biochemical activity. Bacteria can basically grow in three different modes: free (or dispersed) bacteria, floc-forming bacteria and filamentous bacteria. Most bacteria in activated sludge are rod-shaped and gram-negative. Other types of microorganisms are fungi, algae, protozoa, rotifers, crustaceans and viruses. Algae and fungi have a minimal function on the degradation of organic material. Depending on the process used and the quality of the influent wastewater, different types of microorganisms are favoured (competition between different microorganisms for the available nutrients). Factors such as dissolved oxygen concentration, pH, temperature, sludge age and wastewater composition affect the composition of the microflora in the activated sludge. The population of microorganisms in the activated sludge is constantly changing with the influent wastewater composition. This makes the activated sludge process so adaptive.

The bacteria are the microorganisms which carry out the degradation of organic material but other microorganisms are important for an effective treatment. Microorganisms like protozoa and rotifers consume small biological flocs and dispersed bacteria and keep the effluent clear. The presence of higher organisms can also be a valuable tool to estimate the performance of the activated sludge process. High concentrations of protozoa usually indicate that the activated sludge is in balance.

It is desirable that the microorganisms degrade the organic material in the wastewater as fast as possible but they must also be able to form large and strong flocs which is necessary for an effective separation of the biomass. The solids retention time is an important parameter in regulating the flocculation process. It is well known that a certain minimum solids retention time (a low food to microorganism ratio) is necessary for the bacteria to start to excrete extracellular polymers which promotes the flocculation process (see 6.4). A too long sludge age can, however, cause other problems like bulking sludge. Pin-point flocs (very small flocs) are generally found at very long or very short sludge ages. A highly loaded plant (ie a short sludge age) has a larger number of viable cells than a low loaded plant.

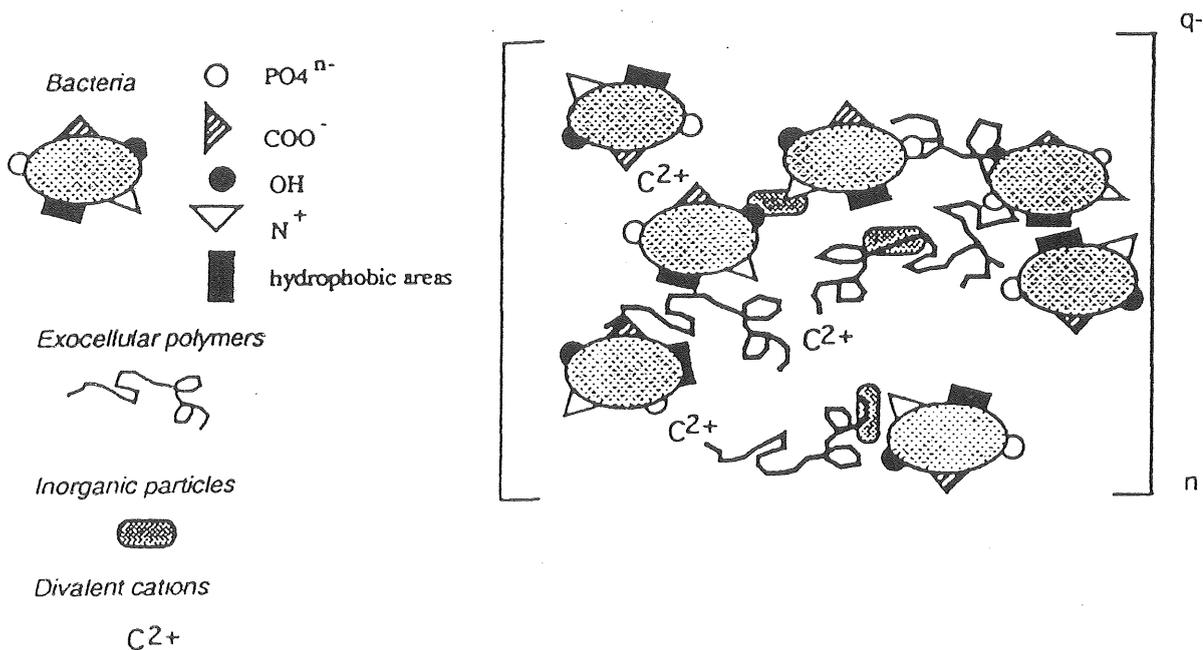
The mechanisms behind the flocculation process are not fully understood but there are a few probable ones: the bacteria excrete extracellular polymers which join the cells together by means of divalent cations; the cells are negatively charged and they are joined together by cations; some bacteria form long filaments onto which other bacteria can adhere. This is described in more detail in chapter 6.

Only the dissolved part of the organic material can be directly taken up by the microorganisms. The rest of the organic material is as a first step adsorbed onto the sludge flocs (biosorption). In the next step is the organic material broken down into smaller constituents by means of enzymes outside the cells. This process is slower than the removal of the compounds supplied by the

wastewater (by means of biosorption). How well a certain sludge adsorbs the organic material depends on the structure of the activated sludge flocs. Pujol and Canler (1992) found that the biosorption increases as the SVI (see chapter 6.1) increases and that anoxic periods negatively affected the biosorption.

## 6. Structure of Activated Sludge Flocs

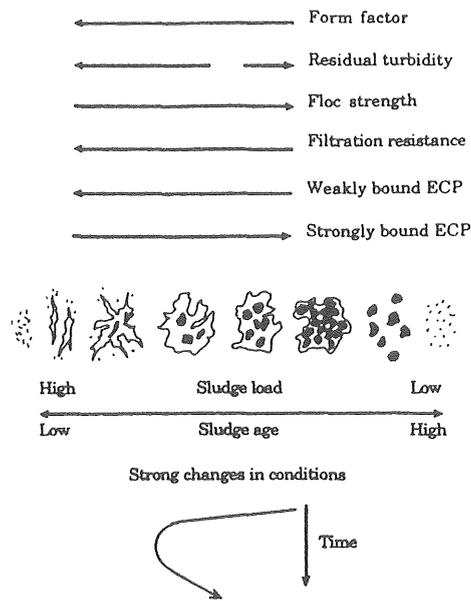
Many models for the mechanism of flocculation have been put forward. The generally accepted models are the polymer bridging model (Busch and Stumm, 1968, Pavoni et al, 1972) and the filamentous backbone model (Sezgin et al, 1978). In both models, the exocellular polymers are believed to maintain the surface charge, but the forces that bind the cells together are different. The polymer bridging model suggests that polymers bind microorganisms together by means of electrostatic forces while the filamentous backbone model suggests that filamentous bacteria create a backbone onto which exopolysaccharide producing microorganisms can attach. This enables particulate and colloidal material to adhere to the activated sludge floc surface. The polymer bridging model is schematically presented in Figure 6.1.



**Figure 6.1.** Schematic presentation of an activated sludge floc (Urbain et al, 1993).

Eriksson et al (1992) proposed a general model for activated sludge properties at different sludge ages or sludge loadings (see Figure 6.2). Floc strength is defined as the ability of the floc to retain good filterability after high shear (small flocs have a less good filterability than large ones). They also pointed out that compact flocs of higher sludge age are very strong and settle faster, but their

surface is too smooth to sweep smaller particles with them during settling.



**Figure 6.2.** A general model for flocs built by floc-forming activated sludge bacteria (Eriksson et al, 1992).

Few studies of the structure of activated sludge flocs can be found in the literature due to difficulties in finding suitable analysis techniques which do not alter the floc structure during handling. Contradictory results are often encountered due to the inaccuracy in the various measurement techniques. Activated sludge flocs have a very complex structure and there are various techniques available to describe their physico-chemical structure. Urbain et al (1993) have made a summary of the most common methods to describe floc characteristics. The various factors normally studied include: amount of filamentous microorganisms, floc size, surface charge, amount of extracellular polymers, amount of divalent cations (e.g.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ), settling velocity, floc strength, floc density and hydrophobicity.

In the following sections the various activated sludge parameters are described in more detail.

## 6.1. Sludge volume index (SVI)

The most common method to evaluate how well a sludge settles and compacts is to measure the SVI. This is the volume occupied by 1 g of sludge after 30 minutes of settling. It cannot be related to the initial settling velocity of the sludge and it varies with the concentration of the initial solids concentration. This is not a scientific parameter (and it does not say anything about the composition of the sludge), but it is a valuable tool in estimating the required flow rate of return sludge to avoid sludge build up in the settler and to notice changes in the sludge settleability. Dick and Vesilind (1969) have made an analysis of what SVI is and what parameters affect its value. They found that parameters like suspended solids concentration, cylinder diameter and height, temperature and stirring affected the SVI. This shows that SVI is a very nonspecific and arbitrary measure of the physical characteristics of activated sludge. It is impor-

tant to note that the settling characteristics in a settling cylinder are not the same as in a full-scale plant and it is meaningless to compare SVI for different plants.

High SVI is usually a result of excessive growth of filamentous microorganisms. A sludge with a SVI > 150 ml/g is generally considered as bulking.

Eriksson et al (1984) made a study to relate the settling properties to the floc structure. They compared the SVI with the SSVI (Stirred Specific Volume Index is measured in the same way as the SVI but with slow stirring) and found that the latter normally gave lower values. The difference varied, however, with sludge type; elongated flocs gave a larger difference than round ones.

Daigger and Roper (1985) made a study to correlate batch settling data with SVI to be able to develop a more practical method to estimate activated sludge settling characteristics. A relationship was determined which related SVI and suspended solids concentration to the initial settling velocity. This simplifies the use of the solids flux theory (see chapter 3.2) in designing secondary sedimentation tanks.

Hultman et al. (1991) proposed that the traditional SVI, which is dependent on suspended solids concentration) would be replaced by more suitable sludge indices to predict settlability such as:

- diluted sludge index (DSVI) in which the sludge is diluted in such a way that the sludge volume is less than 250 ml/l after 30 minutes settling.
- sludge quality index (SQI) where  $SQI = SV/SS$  for  $SV < 300$  ml/l and  $SQI = (200 + SV/3)/SS$  for  $300 \text{ ml/l} < SV < 800 \text{ ml/l}$  ( $SV = \text{sludge volume}$ ,  $SS = \text{suspended solids concentration}$ ).
- stirred specific volume index ( $SSVI_{3.5}$ ) where a standard concentration of 3.5 g/l is used.

The SVI has been criticized because of its inconsistency in performance. It could probably be of much more value if a standard method was used which minimize the effect of cylinder diameter, suspended solids concentration etc.

## 6.2. Filamentous bacteria

Excessive growth of filamentous bacteria gives rise to bulking sludge i.e. a sludge with poor settling properties. Sludge with a SVI higher than 150 ml/g is usually considered as bulking sludge. The filamentous bacteria that causes bulking is a wide and heterogeneous group (Eikelboom and van Buijsen, 1981). The various filamentous microorganisms can grow in different forms; rigid straight filaments that stick out into the bulking solution and coiled filaments that wrap around the flocs to mention some. From this it can be assumed that the settling properties are affected by the type of filamentous microorganism present. Extensive research has been carried out during the last 20 years and even though the knowledge of the control of bulking sludge has increased considerably, lots of plants suffer from this phenomenon.

Different studies have demonstrated that many factors can influence the growth of filamentous bacteria such as low oxygen concentrations, high oxygen concentrations, high sulphide concentration, lack of certain nutrients like N and P, too high or too low sludge loading, high

carbohydrate concentration, high concentration of fatty acids and the feed pattern. Many contradictory explanations can be found in the literature which indicates that filamentous microorganisms are not a metabolically homogeneous group. About 30 different types of filamentous bacteria have been distinguished. One or more filamentous organisms are usually dominating in the activated sludge.

A considerable amount of work has been done within the field of bulking sludge and only a brief summary is found in this report. A breakthrough in the control of bulking sludge was done by Chudoba et al (1973, 1974) and Rensink (1966, 1974). Their works clearly proved that the feed pattern of the plant plays an important role in the occurrence or in the absence of bulking sludge. The completely-mixed activated sludge system was found to lead much more to bulking sludge than the plug-flow of fill-and-draw system. This was explained by the difference in initial organic loading of the sludge flocs. In a completely-mixed system the organic material of the wastewater is totally mixed with the whole contents of biomass in the aeration tank. This creates a low loading of organic material of the sludge flocs. In a plug-flow system, the wastewater meets the return sludge at the inlet end of the aeration tank, which creates a high loading of the sludge and the sludge flocs are deeply penetrated with organic material. The high substrate concentration at the inlet of the aeration tank stimulates the growth of floc forming bacteria.

The activated sludge process is a complex system consisting of different types of bacteria and other microorganisms. The bacteria have different growth and substrate uptake rates and they have abilities to use different sources of carbon and energy and different electron acceptors in biochemical reactions. In the conventional activated sludge process, which is operated only under oxic conditions the composition is mainly decided by the difference in growth and substrate removal kinetics (kinetic selection). The oldest and best known bio-kinetic model for the description of bulking phenomena was performed by Chudoba et al (1973). It was proposed that the Monod formulation for the specific growth rate,  $\mu$ , of the filamentous bacteria have lower values for both  $\mu_{\max}$  and  $K_s$  (Figure 6.3);

$$\mu = \frac{\mu_{\max} \cdot S}{K_s + S}$$

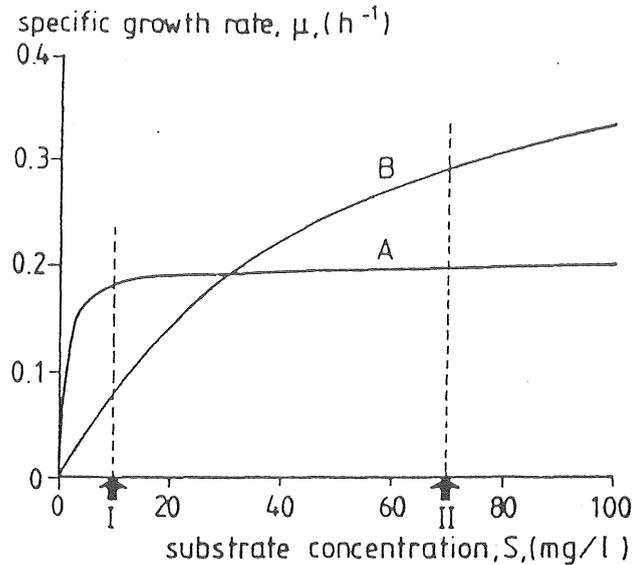
where

$\mu$  = specific growth rate [ $h^{-1}$ ]

$\mu_{\max}$  = maximum growth rate [ $h^{-1}$ ]

$S$  = substrate concentration [g/l]

$K_s$  = Monod constant [g/l]



**Figure 6.3.** Graphical presentation of a relation between growth rate ( $\mu$ ) and substrate concentration ( $S$ ) for two hypothetical organisms: A ( $K_s = 1$  mg/l,  $\mu_{\max} = 0.2$  h<sup>-1</sup>) and B ( $K_s = 50$  mg/l,  $\mu_{\max} = 0.5$  h<sup>-1</sup>).

The filamentous bacteria grow faster than floc forming bacteria at low substrate concentrations due to their higher surface-to-volume ratio while floc forming bacteria have higher growth rates at high substrate concentrations. Thus, the knowledge of the maximum growth rate and half saturation constant for oxygen and various substrates can be used to predict which bacteria have advantage over the others (Lau et al, 1984, Slijkhuis, 1983). It is difficult to determine the actual concentration of the only limiting factor. Wanner (1992) suggested that in mixed cultures cultivated with real wastewater a multiple limitation is quite probable (more than one type of substrate is limiting). This could explain bulking due to low dissolved oxygen levels and/or low levels of certain nutrients. These filamentous bacteria compete with floc forming bacteria even under high substrate concentrations. Several workers have performed experiments to verify the above described theories about kinetic selection. To suppress the filamentous growth by keeping a high substrate concentration in the whole volume of the aeration tank is, however, not possible since that would produce a secondary effluent with poor quality. Instead a high substrate concentration has been created in a part of the plant, a selector, where the sludge and the incoming wastewater is mixed for a short time before entering the main aeration tank. High substrate concentration can be realized in three ways:

- 1) selector
- 2) compartmentalized systems (plug flow)
- 3) sequencing batch reactors

Rensink and Donker (1991) performed pilot plant studies with settled domestic wastewater to study the influence of a selector tank in preventing bulking sludge. A rectangular selector tank which gives rise to a substrate gradient along the reactor could control bulking sludge while a circular selector tank (ie a completely-mixed system) was not able to control bulking sludge. It was also found that well-settling sludge removed COD by biosorption more efficiently than bulking sludge.

Chiesa and Irvine (1985) suggested an other hypothesis of filamentous bulking due to two groups of filamentous bacteria;

- 1) fast-growing, starvation susceptible filaments
- 2) slow-growing, starvation resistant filaments

Continually low substrate concentrations, like in extended aeration systems, was proposed to favour growth of slow growing filaments which have a high substrate affinity and a maximal resistance to starvation. On the other hand, high substrate concentration and low dissolved oxygen concentration would favour the growth of fast growing filaments which have a high affinity for dissolved oxygen. Intermittently fed systems would be unfavourable for filamentous microorganisms in that they have lower substrate uptake rates at high substrate concentrations than floc forming microorganisms. The fast growing filamentous microorganisms would die at extended periods of endogeneous metabolism.

Chudoba (1985) formulated a theory about the difference in accumulation capacities for different microorganisms. The microorganisms which accumulates most of the substrate in the selector or in the inlet part of the aeration system will dominate provided that the regeneration time (the time it takes to break down the adsorbed substrate) for exhaustion of all the substrate is sufficiently long. Therefore it was suggested that a separate sludge regeneration would be advisable. This could explain problems with bulking sludge for selector type systems at very high loadings (Rensink, 1974, Rensink and Donker, 1990). Floc forming microorganisms can accumulate more substrate than the filamentous ones, but when their accumulation capacity is saturated they remove substrate with a rate similar to that of filamentous microorganisms. This would create a mixture of filamentous and floc forming microorganisms in the reactor. The solution would be to find the optimal regeneration time of the sludge to favour the growth of floc formers.

Under oxic conditions the composition of the activated sludge is affected by the wastewater composition and the sludge age of the system as well as growth and substrate kinetics . In general, readily degradable compounds in the wastewater promote the growth of filamentous microorganisms (Wanner, 1992, Jenkins, 1992) like saccharides, alcohols, low fatty acids, long chain fatty acids and amino acids. Inorganic sulphur compounds can also support filamentous growth (Echeverría et al, 1992). Not much information can be found in the literature about the relationship between sludge age and filamentous bulking. In plants operated at very low sludge loadings (typical for nitrifying-denitrifying activated sludge systems because of slow growing nitrifiers) a certain type of microorganisms has been found, so called low F/M filamentous microorganisms ( Gabb et al, 1991). The F/M ratio is called the food-to-microorganism ratio i.e. the amount of organic material divided by the amount of microorganisms present. Literature reviews ( Wanner, 1992, Jenkins, 1992) show that some filamentous microorganisms seem to be able to grow in a wide range of sludge ages, while others grow in a more narrow range.

Most filamentous bacteria are not able to carry out anaerobic respiration ( Wanner and Grau, 1988) using nitrate as the electron acceptor or to use polyphosphate as a source of energy. Jenkins (1991) suggested that anaerobic and anoxic selectors could be used to prevent filamentous growth. Chudoba and Pujol (1994) have written a literature review concerning the design of selectors.

As a summary it can be concluded that the problem with bulking sludge is very complicated due to the big variety of filamentous microorganisms and that many of them can thrive in different environments. Wanner (1994) has made a summary of the most important aspects of filamentous microorganisms.

### **6.3 Surface charge**

Microorganisms acquire a surface charge through ionization of carboxyl and aminogroups (negatively charged at high pH, positively charged at low pH and neutrally charged at the isoelectric point). At the pH for wastewater, the sludge flocs acquire a net negative charge. When a particle is charged, ions of the opposite charge are attached to the surface. The potential at the surface of this cloud of counter ions is called the zeta potential. The zeta potential of solids in suspension is measured in terms of the electrophoretic mobility of the solids (the surface charge is related to the speed at which colloidal particles move in an electric field).

Forster (1968) found a linear relationship between electrophoretic mobility and SVI (i.e. the higher the electrophoretic mobility the higher the SVI). An overgrowth of extracellular polymers would probably increase the negative surface charge and thus increase the SVI. The cells are then bound together by the polymer chains, but they are held in an expanded state.

Steiner et al (1976) studied activated sludges from three wastewater plants to see if there was a relationship between the electrophoretic mobility (which is related to the surface charge) and the SVI. The results showed that there was a linear relationship between surface charge and SVI, but the proportionality constant varied for different sludges. Similar results were obtained by Goodwin et al (1985) as regards SVI, but no relationship was found between zeta -potential and SSVI. The variation in zeta potential was explained to be due to the nature of the surface polymers. Many different biopolymers exist like lipids, proteins, nucleic acids and polysaccharides. Infrared studies showed that a significant part of the biopolymers found were of lipid nature and it was suggested that the surface interactions which influence settlement appear to be in part ionic and in part hydrophobic.

Forster (1971) measured the isoelectric points and the pK values for the surfaces of activated sludge particles with different sludge volume indices at low ionic strength.

The results indicated that the surfaces were of polysaccharide nature, with the most important principal ionogenic monomer being glucuronic acid ( $pK = 3.2$  which would make it fully ionized at the pH of activated sludge).

Pavoni et al (1972) showed that surface potential is not necessarily a prerequisite for flocculation because polymers are able to form bridges between cells either electrostatically or physically. This was confirmed by Chao and Keinath (1979). They measured the electrophoretic mobility for sludge flocs grown under various process loads ( $0.2-2.6 \text{ g COD/g MLSS d}^{-1}$ ) and it did not change and the zeta potential was around  $-24.9 \text{ mV}$ .

Loosdrecht et al (1989) studied the adhesion of bacteria to negatively charged polystyrene. The adhesion was reversible and could be described by the DLVO theory (Derjugin, Landau, Verwey and Overbeek theory). Bacteria and most natural surfaces are usually negatively charged. This leads to a repulsive electrostatic interaction between cells and surface. The degree of interaction depends on the surface potential and the thickness of the electrical double layers (which is inversely proportional to the square root of the ionic strength). If the electrolyte concentration is high or if there are polyvalent counterions present, the electrostatic interaction will be reduced. Microorganisms can adhere in two ways; either by generic physical-chemical forces or with specific surface structures (pili, fimbriae etc). The adhesion by means of physical-chemical forces seems to play a major role when the bacteria are approaching a surface from a long distance (the initial step in bacterial adhesion is often reversible), later the specific interaction becomes important (irreversible interaction).

Zita and Hermansson (1994) investigated the effects of ionic strength on bacterial adhesion and stability of flocs. They found that the floc stability was strongly affected by the ionic strength of the medium and they suggested that the interactions between the floc components can be described by the DLVO theory. The floc stability increased with increasing electrolyte concentration, except when the ionic strength exceeded  $0.1 \text{ M}$ . Detached flocs reflocculated when salt (calcium as well as potassium) was added (decreased turbidity of the supernatant). This indicates that the reflocculation and destabilization of flocs were due to changes in double-layer thickness and not to the bridging effects of multivalent ions. The mechanisms involved in colloid interactions in solid-liquid separation are summarized by Gregory (1993).

## **6.4. Extracellular polymers**

Extracellular polymers (ECP) are compounds excreted by the bacteria and the reason for its production is not fully understood. They play, however, an important role in the flocculation process of activated sludge (Pavoni et al, 1972; Brown and Lester, 1980; Eriksson and Härdin, 1984). ECP can be divided into two categories: polymers in the bulk liquid and polymers attached to the cell surface or incorporated in the flocs. Extracellular polymers consist of high molecular weight compounds ( $M_w > 10,000$ ). It has been suggested that the extracellular polymers at the cell surface are most important for flocculation in that they affect the surface properties of the flocs. The production of extracellular polymers depends on the loading and operation of the plant. Extracellular polymers consist mainly of polysaccharides, protein and nucleic acids. Nowadays it is believed that the ECP are coming from the metabolism and lysis of microorganisms (proteins, DNA, polysaccharides and lipids) as well as from the wastewater

(Urbain et al, 1993).

The most common components of extracellular polysaccharides are summarized by Christensen (1989). Microbial polysaccharides which are positively charged are exceptionally rare; most of them are negatively (and therefore hydrophilic) or neutrally charged. Few have hydrophobic groups (long hydrocarbon chains or aromatic rings).

### *Extraction of ECP*

To be able to measure the amount of exocellular polymers the polymeric material must be extracted from the microorganisms. The problem is to find a method that extracts the polymers you want to investigate. A too strong extraction can cause lysis of the cells which contaminate the sample with intracellular polymers. Various extraction methods such as heat extraction, ultracentrifugation, sonication and chemical extraction have been used. Due to the difference in extraction efficiency, it is difficult to compare literature data.

Forster (1971) extracted polymers from sludge by heat extraction. A chemical analysis showed that the fraction of polysaccharides increased with SVI (10 - 45% for a SVI of 46 - 190, respectively).

Brown and Lester (1980) compared five different bacterial extracellular polymer extraction methods. Steaming treatment was found to be the most effective extraction method for activated sludges. It released a significant quantity of ECP and caused less cell disruption (breakage of cells). Treatment with NaOH was found to be very strong and caused a high degree of cell disruption. Ultrasonication was weaker (the effect is dependent on the sonication intensity) and released low concentrations of extracellular polymers and the degree of cell disruption was low. High-speed centrifugation (33,000 G) was not effective in removing ECP.

Novak and Haugan (1981) performed studies to investigate the efficiency of extracellular polymer removal from activated sludge by means of centrifugation. Different G-values (1,000 - 32,000 G) were tested as well as two different precipitation chemicals: acetone and alcohol. The polymers extracted were fractionated by means of Sephadex dextran gel. The same analysis was performed on supernatant from settled activated sludge without centrifugation. Hardly any difference could be seen between the samples which would indicate that centrifugation does not remove extracellular polymers to a large extent. The authors suggested that the results Pavoni et al (1972) obtained were not due to stripped exocellular polymers but due to the removal of divalent cations from the activated sludge.

Horan and Eccles (1986) developed a three stage purification step to extract exocellular polymers from activated sludge. This procedure yields a polysaccharide fraction free from contaminating protein, nucleic acid and lipid. Exopolysaccharide fractions from five different wastewater treatment plants were compared in terms of monomer composition and molecular weight distribution (obtained by HPLC). All the sludge samples contained exocellular polymers with a weight fraction in excess of 160,000 and one or two distinct fractions of lower molecular weight. Five monomers were detected: glucose, galactose, mannose, glucuronic acid and galacturonic acid. The concentration and nature of such ionogenic polymers present at the surface determined the sludge surface charge to values between -10 to -20 mV.

Sanin and Vesilind (1994) studied the effects of centrifugal force on the extraction of the ECP from activated sludge. They tested different G-values: 2,000, 5,000, 11,000, 14,000, and 16,500.

ECP was removed from the sludge by centrifugation but the removal was not linear with the G-value. There was only a slight increase in extraction efficiency as the centrifugal force increased. Data from this study show that polymers extracted constitute 2% of the total mass of sludge solids. This is surprisingly low. The explanation could be that there are very different ECPs which bond with different strengths; hydrogen bonding (polysaccharides) and hydrophobic bonding (lipids, proteins).

#### *The structure of activated sludge flocs in relation to the ECP*

Li and Ganczarzyk (1990) investigated the structure of activated sludge flocs (larger than 100  $\mu\text{m}$ ) by stabilizing them in a histological tissue. The samples were then sliced into sections of 3-6  $\mu\text{m}$ . It was found that the structure was non-uniform and the extracellular polymers in the flocs varied randomly on the plane of the sections. Extracellular polymers and water were the major components outside the cells within a floc.

Eriksson et al (1992) investigated the sludge properties and the concentration of suspended solids in the effluent in two different lines of an activated sludge plant. Measurements were carried out during a period of one year to be able to follow the seasonal change in sludge composition. Special attention was paid to the snow melting period since the biomass separation usually works less well than. It was found that the residual turbidities were higher during the denitrification period when the sludge age was higher. This was explained by the fact that older flocs are more round and smooth than younger ones and have less ability to sweep fine dispersed material during the settling than more irregular younger flocs. Extracellular polymers were extracted from the sludge by three methods of different strength; centrifugation, treatment in Waring blender and treatment in Waring blender after a pH adjustment to 9.7. It was found that the amount of weakly bound polymers increased with decreasing floc strength. The floc strength was measured as the capillary suction time (the slope of the linear regression line when plotting capillary suction time against agitation time gives information on floc strength). A model for activated sludge floc properties was proposed: since both the cell surfaces and the polymers are negatively charged at neutral pH, the binding between polymers and cell surfaces and between different polymers will be counteracted by electrostatic repulsion unless polyvalent metal ions are present. These cations form electrostatic bridges between the negative groups and are in most cases necessary for bioflocculation to occur. It can be assumed that the outer parts of the floc have lower sludge age and higher cell growth rates than the interior parts due to the diffusional resistances and should thus contain fewer polymers than the interior. This could explain why the outer parts of the flocs are shear sensitive. Older flocs are more round and contain more polymers. It was also proposed that a change in operating conditions could favour new types of organisms and would give the sludge properties of young sludge. After some time the properties will return to those of the calculated sludge age.

Jorand et al (1995) described the complexity of the activated sludge floc structure using four methods: microscopic observation in situ and after staining, optimization of the floc dispersion by sonication (dispersion of cells by means of an ultrasound generator) of pure bacterial strains, analysis of polymers released from sonicated sludges and floc size distribution after different sonication times. The polymers released (37W for 60 s) were mainly proteins, polysaccharides and DNA. Electron microscopy showed that a polysaccharide gel connected the cells together. The raw activated sludge had a continuous distribution of particle sizes (1.2-600  $\mu\text{m}$ ). By measuring the floc size distribution after different sonication times, a model for floc structure was suggested. The model has three structural levels of microflocs, which are primary particles 2.5  $\mu\text{m}$  in size, secondary particles (13  $\mu\text{m}$ ) linked together by extracellular polymers and forming

tertiary structures having a mean diameter of 125  $\mu\text{m}$ .

***The effect of ECP on the settling properties of activated sludge***

McKinney (1953, 1956) was the first to find a relationship between the accumulation of polysaccharide material and bacterial flocculation. He postulated that polysaccharide material in the cell wall reduce the critical potential of the cells so that flocculation could occur. Much work has been done within this field throughout the years. In recent years the most plausible flocculation model is the polymer-bridging model. Long-chain polymers are believed to form bridges between bacterial cells to form large aggregates. Eriksson et al (1984) proposed a unifying model of flocculation. It suggests that during the initiation of flocculation, exocellular polymers are responsible for bridging the distance between electrostatically stabilized cells to form weak, elongated flocs. Up to a certain level polysaccharide synthesis produces stronger flocs by binding the cells more firmly. A further synthesis causes a dispersion of the cells. This would indicate that two types of polymers are present: binding and non-binding. The polymer that causes the best flocculation has a high molecular weight and contains a negative charge. Large polymers can bind independently of direction which leads to round flocs. A change in the environment surrounding the cells can alter the metabolism and produce polymers of different sizes.

Pavoni et al (1972) performed batch-tests to study the effect of extracellular polymers on the bioflocculation of activated sludge. The results showed that bioflocculation took place only after that the microorganisms had entered the endogenous growth phase. The ratio exocellular polymer:microorganisms increased during growth. Removal of extracellular polymeric material (by means of centrifugation at 32,500 G) from well-flocculated bacterial suspensions followed by subsequent resuspension of the harvested cells resulted in a stable suspension. Readdition of the extracted exocellular polymers resulted in reflocculation. Exocellular polymers extracted from activated sludge flocculated also stable inorganic dispersions of kaolinite, silica and alumina.

In a study by Magara et al (1976), it was found that the settlability deteriorated as the amount of extracellular polymers as well as PHB (poly- $\beta$ -hydroxybutyrate, which is a lipid) increased. That means that the settling characteristics improved by decreasing the electrophoretic mobility due to the disappearance of extracellular polymers and PHB.

The settlement characteristics of sludge obtained from full-scale plants were studied by Forster (1985a). The results showed that there were certain combinations of nutrients that were more likely to produce poor settlement (high SVI). Surface polymers (extracellular polymers) were extracted from the sludge by heat and determined by a gel filtration with an exclusion limit of 100,000. Three major fractions of polymers were found;  $\geq 100,000$ ,  $< 100,000$  and one or more minor fractions, and the settling deteriorated as the weight of polymer increased.

Contradictory results were found by Goodwin et al (1985); the amount of polysaccharides in the extracted exocellular polymers increased as the settlement improved. However, the amount of lipids in the exocellular polymers increased as the SVI increased. The ECP was extracted by means of heat at temperatures ranging from 50 to 100  $^{\circ}\text{C}$ . The lipid and polysaccharide fractions were less sensitive to temperature differences than the protein fraction. The conclusion of this was that the choice of extraction temperature can determine the balance of the polymers obtained. It was further suggested that the interactions that affect the settling properties are partly ionic (related to the polysaccharides) and partly hydrophobic (related to the lipids).

Morgan et al (1990) compared the biopolymers extracted from activated and anaerobic sludges. It was noticed that activated sludge produced much more ECP than the anaerobic sludge but they contained less protein. The activated sludge flocs were also more electronegative than the anaerobic ones.

Wahlberg et al (1992) measured the amount of extracellular polymers in situ. Ruthenium red (RR) adsorbs selectively to acidic polysaccharide. This has the advantage of not destroying the cells. A series of flocculation tests were performed at full-scale plants. The turbulence level was kept constant, but the flocculation time was varied. The samples settled for 30 minutes and the turbidity was measured on the supernatant and ECP was measured in the sludge. The data was then fitted to a differential equation describing floc aggregation and breakup. The floc breakup decreased as the concentration of ECP increased up to a threshold value after which it was constant. The number of primary particles (very small sludge flocs) also showed a declining trend with ECP.

Urbain et al. (1993) found a positive relationship between SVI and amount of extracellular polymers, i.e. high concentrations of extracellular polymers resulted in a worsening of sludge settlability.

Andreadakis (1993) determined the amount of carbohydrate in activated sludge taken from a bench-scale plant colorimetrically to 6 - 18% and it could not be correlated to sludge settlability.

Many plants are rebuilt to be able to perform nitrogen and phosphorus removal. This might change the composition of the biopolymer matrix. Frølund et al. (1994) compared biopolymer characteristics from a traditional and an advanced activated sludge treatment plant. An ion exchange resin was used to extract biopolymers. There were differences in chemical composition (protein, polysaccharide and uronic acid) of the total sludge but not in the extracted polymers. The biopolymers were analysed with high pressure size exclusion chromatography and it was found that the bio-polymers from the advanced treatment plant contained two fractions of large and hydrophobic compounds. These peaks could not be found in biopolymers from the traditional wastewater plant.

This literature review shows that many contradictory results have been obtained due to the difference in extraction methods. In earlier studies, it was believed that extracellular polymers bound chemically to the surface of bacteria, while later studies show that a more plausible explanation is that polymers form bridges between bacteria through multivalent cations. It seems as though the settling properties of activated sludge improve with ECP concentration up to a certain level where the settling properties begin to deteriorate.

Many different polymers have been found in extracellular polymers, such as polysaccharides, proteins, nucleic acids, glycoproteins and humic acids, but most emphasis has been put on polysaccharides. The other polymers are probably also of significant importance.

## 6.5. Polyvalent metal ions

That metal ions interact with activated sludge is a well known fact. This is important in removing metals from the wastewater but it is also believed to play an important role in the formation of activated sludge flocs. Steiner et al. (1976) found that polyvalent ions were important for the floc structure because of their capability to form bonds between exopolymers, probably by binding to carboxyl and hydroxyl groups. Different types of adsorption isotherms were obtained with cations like copper, calcium and cobalt and they had a higher affinity for extracellular polymers alone than for the rest of the sludge.

Forster (1985) studied the binding of polyvalent metal ions (copper, zinc, nickel and chromium) to extracellular polymers extracted from activated sludge from full-scale plants with different SSVI. The binding properties of the extracellular polymers to polyvalent metal ions were examined by two methods: gel filtration and equilibrium analysis. The results showed that the number of binding sites for metal ions varied randomly with SSVI. Cu, Zn and Cr behave similarly but the binding of Ni depends on the settlement properties of the activated sludge.

Various data in the literature indicate that  $\text{Ca}^{2+}$  is the most important cation.  $\text{Ca}^{2+}$  can be extracted from activated sludge in various ways: decreasing pH, addition of EDTA (Kakii et al. 1990, Eriksson et al. 1991) and by a  $\text{Ca}^{2+}$ -specific chelant (forms a complex with  $\text{Ca}^{2+}$ ), EGTA (Turakhia et al. 1983). Removal of  $\text{Ca}^{2+}$  gives rise to a more turbid supernatant.

Eriksson and Axberg (1981) added  $\text{CaCl}_2$  to a sample of a model system of washed E. Coli B harvested at the end of the logarithmic phase at two different pH values; 7 and 10. At pH 10 the rate of sedimentation increased rapidly with increasing  $\text{Ca}^{2+}$  concentration while at pH 7 it decreased for  $\text{Ca}^{2+}$  concentration up to 0.001 M and then increased at higher concentrations. Addition of EDTA decreased the settlability at both pH 7 and pH 10 due to strong complex bilding of  $\text{Ca}^{2+}$  to EDTA. The decrease in sedimentation rate at low concentrations of  $\text{CaCl}_2$  at pH 7 was explained by a change of negatively charged polymers from an elongated to a coiled conformation and thus diminishing the bridging ability. At higher  $\text{Ca}^{2+}$  concentrations there will be a recharging of the polymers which again become stretched out to be more effective for bridging.

Eriksson et al. (1991) studied the electrostatic interaction between bacterial surfaces, extracellular polymers and polyvalent metal ions. This was done by adding different concentrations of EDTA to activated sludge samples from full-scale plants. A strong complexing agent such as EDTA may have a dispersing effect on microbial flocs. EDTA was found to have a significant effect on the sedimentation velocity. The settling tests indicated that the polymers interact with the primary flocs by means of metal ions and that this takes place at the outer surface of the primary flocs. It was also found that a combination of stirring and addition of EDTA created larger and more compact sludge flocs.

Bruus et al. (1992) found that the extraction of  $\text{Ca}^{2+}$  from activated sludge flocs led to an increase in the number of small particles and thus a decrease in dewaterability.  $\text{Ca}^{2+}$  was removed by an ion exchange process where  $\text{H}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  or  $\text{Mg}^{2+}$  served as counter ions, or by addition of EGTA. Addition of  $\text{Cu}^{2+}$  improved the filterability of activated sludge at the same time as  $\text{Ca}^{2+}$  was released. This was explained by the fact that the  $\text{Ca}^{2+}$  ions are substituted in the exopolymer structure by  $\text{Cu}^{2+}$  ions which results in a more stable floc structure. The initial concentration of

$\text{Ca}^{2+}$  in the thickened sludge (12-14 g MLSS/l) was 15 mM. 10% of the  $\text{Ca}^{2+}$  could be analysed in the supernatant, after addition of 1% (w/v)  $\text{La}^{3+}$  16% could be analysed in the supernatant, 36% was released after addition of  $\text{K}^+$  or  $\text{Na}^+$ . This indicates that the affinity of the sludge matrix towards the added cations is relatively small. When  $\text{Cu}^{2+}$  was added about 50 % of the  $\text{Ca}^{2+}$  in activated sludge was released (the high affinity of  $\text{Cu}^{2+}$  to the sludge matrix should be able to extract all  $\text{Ca}^{2+}$  from the ECP).

It seems clear from this literature review that polyvalent cations play an important role in the flocculation process of activated sludge. Information is, however, missing about how they accumulate in the sludge and if they have affinity for special polymers. It should also be interesting to know if the amount of divalent cations is high enough in wastewater to cause an optimal flocculation (the concentration of  $\text{Ca}^{2+}$  varies probably within a wide range due to differences in hardness of water etc).

## 6.6. Floc size and size distribution

The variation in particle size, density and porosity has a large impact on the settling properties of activated sludge suspensions. It is also an important physical factor in the dewatering process of sludge. Characterization of a suspension by its particle size distribution is important to be able to evaluate the performance of a certain separation technique. The particles can be characterized in terms of diameter, surface area and volume. Many of the chemical and microbiological contaminants in wastewater are adsorbed on the surface of particles. The relative distribution of these contaminants between different size fractions of the particulates depends on the surface properties, surface chemistry, particle shape and size distribution of particles. The particle characteristics are further influenced by the addition of chemical flocculants as Al and Fe salts (Boller, 1993), which do not only change the surface chemistry but also the particle size, density and number. In the literature little data can be found with respect to floc characterization after the addition of chemicals.

It is, however, difficult to measure the size distribution of flocs since they are heterogeneous and the size range is very broad. Difficulties are also encountered during sampling to avoid reflocculation and other physical alterations of the activated sludge flocs. Various methods for particle size measurements are available. The different techniques are summarized by Kavanaugh et al (1980). Different techniques characterize the particles in different ways.

**Table 6.1.** Summary of different size measurement techniques.

Measuring principle	Equivalent size measured	Size limits minimum ( $\mu\text{m}$ )	R
Electron microscopy	statistical length	0.001	50-200
Optical microscopy	statistical length	0.3	40
Coulter counter (electrical sensing zone method)	volume diameter	1	20
Light scattering laser method	cross section diameter	1	10-50
Light obscuration	cross section diameter	1	50-60

R = ratio of maximum to minimum size for single sensing element, or single magnification.

Leentvaar et al (1983) measured the size of ferric hydroxide flocs by a photographic method. A batch coagulation-flocculation tank was placed on a platform in such a way that pictures were taken from the front side of the tank. The advantage by this technique is that the measurement takes place in-situ.

Karr et al (1978) developed a method to fractionate activated sludge flocs by size. The sludge was filtered through meshes and membranes of different sizes. The drawback with this method is that smaller flocs are adsorbed onto larger ones and removed already at the mesh with a large pore size.

Li and Ganczarczyk (1987) introduced a multi-exposure photographic (stroboscopic) method for measurements of settling velocity and size of activated sludge flocs. The smallest flocs investigated were about 50  $\mu\text{m}$ . With the cross-section diameter as a criterion, the settling velocity was linear with floc size. Using the longest dimension as the size criterion, the settling velocity satisfied both a linear function and a power function (0.55 power relationship). This is not consistent with Stokes' law which states that particle settling velocities are related to the second power of particle diameter in a laminar flow. In a few cases, the floc settling velocities did not increase as the floc size increased. This was explained by the fact that flocs with irregular shapes settle with a lower velocity. The data from the measurement of size and settling velocity was used to calculate the porosity of the activated sludge flocs, which was found to increase at two different rates with the increase in the floc longest dimension.

Magara et al (1976) used a jar tester to determine photographically the size and settling velocities of activated sludge flocs. A linear correlation was found between size and density for a given organic load, ie the density decreased with increased size. Tambo and Watanabe (1979) studied the size and settling velocity of aluminium flocs by means of a quiescent water column with a

camera connected to it. The floc density was calculated with a modified Stokes' equation.

Zahid and Ganczarczyk (1990) studied the suspended solids in the effluents from biological filters. Size indices and shape factors were determined with the use of an image analysis system. The samples were solidified in agar to prevent alteration during storage. Particles of a size less than 10  $\mu\text{m}$  were not considered. The settling velocities of individual particles were measured in a quiescent water column using a multiexposure photographic technique (Li and Ganczarczyk, 1987). The particles wet density was calculated using Stoke' law of free settling. Particle porosity was calculated according to Tambo and Hozumi (1976); Tambo and Watanabe (1979) which assume that the flocs consists of two parts, a solid part and a void (water) part. The calculations showed that particles larger than 100  $\mu\text{m}$  have a more open structure while smaller particles have a porosity that is much dependent on their sizes.

Li and Ganczarczyk (1991) analysed samples of mixed liquor from five conventional wastewater plants for size distribution. Flocs smaller than 10  $\mu\text{m}$  were measured by a Coulter counter and larger ones by an image analysis system. The size distribution expressed as frequency of occurrence across the whole spectrum correlated well with the power-law and Rosin-Rammler models and flocs larger than 10  $\mu\text{m}$  fitted best the log-normal model. It was also found that flocs larger than 50  $\mu\text{m}$  are the major source of surface area, volume, and mass although they are fewer than the smaller ones. Flocs smaller than 2  $\mu\text{m}$  can be ignored in terms of surface area and volume but can contribute with up to 18 % of the mass provided by the activated sludge.

Andreadakis (1993) made a study to evaluate physical and chemical characteristics of activated sludge such as floc size, density, specific surface, carbohydrate content, dehydrogenase activity and settlability. Seven parallel bench scale activated sludge units operated under different sludge ages (1.1-17.4 days) were compared. 85 % of the flocs were in the size range 10-70  $\mu\text{m}$  (measured by a Coulter Counter) with median values between 35-45  $\mu\text{m}$  and with a density of 1.015-1.034  $\text{g cm}^{-3}$ . A strong correlation between floc density and size was noticed. Sludge settlability (non-filamentous sludges) was correlated to floc size, density and specific area but not to the carbohydrate content which varied between 6-18 %. This could be explained by the fact that the amount of exocellular polymer is not the major factor in sludge bulking but the properties of the polymers.

Li and Ganczarczyk (1993) made a study to correlate the power-law and log-normal floc size distribution models (Li and Ganczarczyk, 1991) with the values of process operation factors for five, full-scale conventional activated sludge wastewater plants. Special emphasis was put on the formation of primary particles, i.e. dispersed bacteria and very small flocs. The process operation factors analysed were: food to microorganisms ratio, dynamic sludge age, mean velocity gradient, hydraulic retention time, dissolved oxygen, mixed liquor suspended solids and mixed liquor temperature. It was found that the process operation factors affect the floc size distribution in a very complex way, but sludge loading was the most statistically significant factor influencing the frequency of occurrence of primary particles (< 2 $\mu\text{m}$ ).

Ganczarczyk (1994) subjected activated sludge mixed liquor from full-scale wastewater plants to free settling tests according to Li and Ganczarczyk (1987). They correlated different shape factors such as longest dimension, longest diameter perpendicular to the settling direction, perimeter and the flocs area equal to the settling floc projection (Allen, 1981) to the settling velocity. Linear regression models for the evaluation of free settling velocity as a function of their size values produced for the particular flocs high correlation coefficients ( $r^2$ : 79.22-81.41%).

Multiplicate regression models gave an almost linear relationship, but the  $r^2$  was lower (71.79-74.74%). The  $r^2$  factor was improved (92.72-94.56%) by incorporating shape factors. The conclusion of this is that the shape factors affect the free-settling velocity to a large extent. Equivalent diameter gave best correlation then longest dimension perpendicular to the settling direction and longest dimension. Small flocs have a shape factor close to one (for a sphere the shape factor is equal to one) and as the size increases the shape factor decreases.

More emphasis has been put on the size distribution of activated sludge the last years. It seems to be a valuable tool in following what happens in the different treatment steps. The major obstacle is to find suitable measurement techniques that are easy to use. Activated sludge flocs are very irregular and it is difficult to define their size.

### **6.7. Floc porosity and density**

The porosity of the activated sludge floc is believed to affect the diffusion of compounds like oxygen and substrates within the floc.

Li and Ganczarczyk (1988) investigated the flow through activated sludge flocs. They compared the settling velocity of flocs containing a biomass carrier with flocs without and they found that flocs without biomass carrier did not settle with a velocity lower than those with. It was proposed that microbial aggregates are so porous that they might be permeable to fluid flow within certain shear rates or during gravity settling.

Determining the density of activated sludge flocs is difficult since the floc structure is very fragile and can be altered during measurement. Dammel and Schroeder (1991) used density gradient centrifugation to determine activated sludge floc density. A fluid column with increasing density with depth was constructed and the sedimentation rate was increased by using a centrifuge to increase the gravitational force. They measured the densities to 1.02-1.06 g/ml.

The value is, however questionable (probably a bit lower in reality) since the risk is high that the floc structure is compressed during centrifugation.

### **6.8. Hydrophobic and hydrophilic nature of activated sludge flocs**

A molecule that is charged or polar is hydrophilic (strongly adhering to water) and a nonpolar molecule is hydrophobic (not readily mixing with water). Bacteria and other microorganisms like algae, protozoa and viruses can be considered to be hydrophilic biocolloids (Tenney and Stumm, 1965). Within the pH range of interest for wastewater treatment, the microorganisms carry a net negative charge. It is difficult to study the colloidal characteristics of bacteria because there are so many types with different surface composition. Hydrophobicity is difficult to measure in sludge flocs since most of the methods need dispersed cultures.

Loosdrecht et al (1987) studied the relationship between physicochemical surface parameters and adhesion of bacterial cells to negatively charged polystyrene. Both cell surface hydrophobicity

and electrokinetic potential influenced the cell adhesion. The cell surface characteristics seemed to be influenced by the growth conditions: at high growth rates the surface became more hydrophobic.

Urbain et al (1993) proposed a model for settlability of activated sludge describing the hydrophobic and hydrophilic interactions inside the biological floc. They assumed that a hydrated structure such as a activated sludge floc has internal hydrophobic bondings which are involved in the flocculation process. It was found that an increase in ECP content (hydrophilic) negatively affected the sludge settling properties, while intrafloc hydrophobic interactions improved the settling properties.

Jorand et al (1994) studied the hydrophobicity of activated sludge flocs. Their study shows that hydrophobic bacteria coexist in the flocs with hydrophilic bacteria which seem to release high quantities of extracellular polymers. Addition of easily assimilable nutrients like glucose to the activated sludge does not change hydrophobicity of the bacteria but increases both exocellular polymer production and SVI (exocellular polymers were extracted by sonication). The results indicate that certain strains from activated sludge preserve their surface properties (hydrophobicity/hydrophilicity) during the whole growth phase while others change properties during growth.

## **6.9. Influence of wastewater pollutants on settling characteristics of activated sludge**

Pollutants in the wastewater can directly affect the settling and flocculation characteristics by adsorbing onto the activated sludge flocs and indirectly by affecting the bacterial metabolism. Some of the pollutants are not degraded in the aeration basin and pass into the sedimentation basin dissolved or adsorbed onto flocs.

Eriksson and Axberg (1981) studied the influence of different wastewater pollutants on flocculation and sedimentation of a model system containing *E. Coli* B. Addition of  $\text{CaCl}_2$  (hardness) increased the sedimentation rate. An effect similar to that of  $\text{Ca}^{2+}$  was found for TTAB (tetradecyltrimethyl ammonium bromide) which is a cationic surfactant. The effect of complexing agents was tested by adding EDTA. At pH 7 the sedimentation rate increased as the concentration of EDTA increased. The effect was smaller when  $\text{CaCl}_2$  was added since it binds to EDTA. At pH 10 EDTA strongly inhibits sedimentation and the suspension becomes viscous. The addition of  $\text{CaCl}_2$  decreases the effect. A similar effect was found for humic acid which is a stronger complexing agent than EDTA. The effect of non-ionic surfactants was studied by adding Berol 09 and Berol 08. Both caused an increase in sedimentation rate and the electrophoretic mobility of the suspension decreased (stabilized system). The addition of sodium tripolyphosphate and sodium perborate (orthophosphate) increased the sedimentation rate. The effect of orthophosphate was however, smaller. Sodium silicate (hydrophilic and negatively charged polymer) stabilized the system electrostatically as well as sterically and the sedimentation rate decreased with increasing concentration. Bicarbonate increased the sedimentation rate and lowered the electrophoretic mobility. The effect of polysaccharide was investigated by adding carboxy methyl cellulose which increased the sedimentation rate at both 10 and 100 ppm. A higher concentration would probably stabilize the system. The effect of

colloidal particles was studied by adding SBR-latex and it was found to decrease the sedimentation rate (anionic surfactant which stabilize the bacteria). Soya bean oil and lecithine emulsions had a weak stabilizing effect on the suspension.

New, 'environmentally friendly' washing powders etc change the composition of the wastewater and its impact on the settling properties of activated sludge is probable interesting to study in the future.

## 7. Process Parameters Affecting Settling Characteristics

In practice, the operation parameters affecting the performance of the secondary settler can be divided into two major groups:

- 1) factors affecting the floc size and structure in the aeration tank
- 2) factors affecting the sedimentation and flocculation in the secondary settler

The size and structure of activated sludge flocs in the aeration tank can be affected by: solids retention time (sludge age), aeration basin hydraulic regime, hydraulic retention time, reactor configuration, dissolved oxygen concentration, turbulence due to the aeration, concentration of suspended solids, flow rate of return sludge and type of pump used for return sludge (some types of pumps expose the sludge flocs to high shear forces). Factors such as surface overflow rate, suspended solids concentration and retention time affect the sedimentation in the secondary settler.

### 7.1. Solids retention time (sludge age)

Bisogni and Lawrence (1971) studied the relationship between biological solids retention time and the settling characteristics of activated sludge. A series of bench-scale activated sludge units fed with synthetic wastewater and with internal microbial solids recycle were operated at solids retention times between 0.25-12 days. This study was restricted to non-filamentous sludge. Based on the total biomass in the effluent, the best overall solids removal occurred at solids retention times of 4-9 days. At very short solids retention times the effluent contained dispersed growth while at long solids retention times it contained pin-point flocs and small flocs. Similar results were obtained by Chao and Keinath (1979). Low SVI occurred at sludge ages above 5 days and near 2 days. Within the range 2-5 days Zoogloal bulking was noticed and at very short sludge age (<1.9 days) filamentous bulking occurred. The effect of process loading intensities (g COD/g MLSS d<sup>-1</sup>), i.e. the F/M ratio, on SVI was also studied. High SVI was found in the range 0.6-1.3 g COD/g MLSS d<sup>-1</sup> and beyond 1.8 g COD/g MLSS d<sup>-1</sup>. This was caused by excessive growth of filamentous bacteria. The effluent concentration of suspended solids increased with process loading intensity. This was explained by the fact that at low process loadings the production of extracellular polymers is high enough to allow efficient aggregation and will result in low levels of dispersed bacteria.

Lovett et al (1983) fed lab-scale reactors with a synthetic wastewater containing meat extract to evaluate the effect of sludge age on the settlement characteristics. A maximum in SVI was found at a sludge age of about 8 days. As the sludge age was further increased, the SVI decreased. The degree of dispersion increased dramatically as the sludge age was kept below 8 days and at 4 days it was 35 %. When the sludge age was increased beyond 8 days the degree of dispersion decreased with increased sludge age.

Results from different experiments are difficult to compare with each other because of the variation in wastewater composition. This produces different amounts as well as different types of filamentous microorganisms which can mislead the interpretation of the effect of sludge age.

Pipes (1979) investigated the settlability of activated sludge collected from several full-scale wastewater plant over a period of 12 years. Special attention was focused on the formation of pin-point flocs. These are very small flocs which cannot settle out in the settler. They were observed at low organic loading ( $< 0.2 \text{ d}^{-1}$ ). Extreme cases of bulking sludge could also be noticed at low organic loads ( $< 0.2 \text{ d}^{-1}$ ). These both phenomena were not observed simultaneously. Factors such as low dissolved oxygen levels, low pH, and toxic shock loads were found to produce pin-point flocs.

Cashion and Keinath (1983) made a statistical analysis of the effect of three process parameters (solids retention time, hydraulic retention time and clarifier overflow rate) on the clarification efficiency. A small pilot-scale activated sludge plant was fed with domestic wastewater. A statistical analysis of the results showed that two operational regions exists in which low effluent suspended solids values can be attained; low solids retention time (ca 2 days) and high hydraulic retention time (ca 12 hours) or high solids retention time (ca 8 days) and low hydraulic retention time (ca 4 hours)

Knocke et al (1986) studied the effects of mean cell residence time and particle size distribution on activated sludge vacuum dewatering characteristics. An automated particle size counting system was used. Particles in the range 5-100  $\mu\text{m}$  were measured. Sludge characteristics were monitored from both lab-scale (one fed with domestic wastewater and one with synthetic wastewater) and full-scale wastewater treatment facilities. As the mean cell residence time increased to more than 4 days the particle size distribution changed dramatically, to larger particles. At each cell residence time considered, the size of the particles produced in the primary effluent treatment system was always smaller than in the synthetic feed systems. The characteristic size distribution in the full-scale system was significantly smaller than in either of the lab-scale treatment systems. Polymer conditioning studies were conducted with a high molecular weight cationic polymer to improve the dewatering rates of waste sludge samples. In general, as polymer dose increased, the characteristic particle size distribution increased (to larger particles).

Contradictory results can be found in literature concerning the relationship between growth of filamentous bacteria and sludge age (see section 6.2.). At very long sludge ages a special type of filamentous bacteria can be found, so called low F/M filamentous microorganisms (Gabb et al, 1991).

Sheintuch et al (1986) proposed a kinetic model that describes the production of polymers, biomass and inerts in an activated sludge system. In batch reactors fed with synthetic wastewater, the polymers are produced during endogenous respiration. The model could well describe the variation in substrate, biomass and polymer concentration. When the model was applied to a continuous reactor, the polymer concentration increased with sludge age. This was also confirmed experimentally.

Most studies into the affect of organic loading on the physical properties of activated sludge have concentrated on the settlability and the sludge volume index. The floc sizes and size distributions were not measured. In recent research, more attention has been paid on the sizes of the flocs. Li and Ganczarzyk (1993) studied the influence of treatment operation conditions on the size distribution of activated sludge flocs. They found that the organic loading and the dissolved oxygen concentration per unit of organic loading were the two most significant factors influencing the size distribution of the activated sludge flocs.

Barbusiński and Kościelniak (1995) studied the variability in average floc size above 10  $\mu\text{m}$  (microscopic observation) and floc size distribution in response to the degree of organic loading. Under the conditions of dynamic loading changes the distribution of floc sizes larger than 10  $\mu\text{m}$  could be approximated by a log-normal model. The size of the flocs increased with the value of the loading. Long term loadings of the activated sludge caused much larger disturbances to floc size distribution than more rapid short-lasting changes.

## **7.2. Mixed liquor suspended solids concentration**

Tuntoolavest et al (1983) made a statistical analysis to evaluate the effect of solids retention time, mixed liquor suspended solids concentration, sludge recycle rate and the turbulence level in the aeration tank on the amount of suspended material in the effluent. The mixed liquor suspended solids concentration (MLSS) was found to be the factor which affected the effluent quality the most. This confirms an earlier study by Pflanz (1969).

Chapman (1983) studied the influence of process variables (MLSS concentration, settler feed flow rate and under flow rates, air flow rate to the aeration tank proceeding the settler, the depth of the inlet feedwell, the settler sidewater depth and the speed of the sludge collection arm). A factorial experiment was carried out on a pilot-scale settler. The MLSS concentration was the factor that most affected the occurrence of effluent suspended solids while a change in air flow rate had no significant effect. A deep sedimentation tank with a thick sludge blanket produced a clearer effluent since it promotes the entrappment of small flocs and dispersed bacteria in the larger flocs. In a later study (Chapman, 1985), the effect of transient loading was investigated. Disturbances were introduced to the rate of flow and solids concentration of mixed liquor pumped to a pilot-scale settler. The results of the repeated step changes in flow rate showed that both the level and variability of the concentration of suspended solids in the effluent increased as the flow rate increased. It was also found that the settler responded faster for a step increase than for a step decrease. The response to the change in flow rate was non-linear which suggests that turbulence is generated in the settler which dampens out slower than it is generated.

### 7.3. Degree of turbulence

Much work has been made in the past to relate the degree of turbulence to the amount of dispersed bacteria in the effluent. Parker et al (1971) suggested that small-scale eddies (within the same scale as the floc; the viscous dissipation subrange) caused floc break-up while larger eddies could promote flocculation. The addition of a mildly stirred flocculation step (operated at detention times of 20 to 30 minutes) between the aeration tank and the clarifier provided an environment that encouraged aggregation of smaller flocs into larger ones. A theoretical relationship was developed to describe the kinetics of suspended solids removal as a function of intensity and time of agitation during flocculation. The removal efficiency from mechanical flocculation varied for different sludges. This could not be explained.

Koníček and Burdych (1988) studied how different aeration systems in an aeration tank affected floc cohesion. It was found that for diffused air aeration the degree of particle disintegration is lower than for mechanical aeration. Mechanical flocculation tests of activated sludge were performed and the number of particles per volume in the supernatant after 30 minutes settling was measured for various G-values. Particles in the size range 2-65  $\mu\text{m}$  were measured using a Coulter Counter. For sludge from diffused air plants, the number of small particles ( $<3,22 \mu\text{m}$ ) in the supernatant decreased by 35 % at low G-values ( $46 \text{ s}^{-1}$ ), but increased by approximately 40 % at high G-values ( $296 \text{ s}^{-1}$ ). For sludge from mechanically aerated sludge, 60 % of the supernatant particles were removed. High G-values did not cause floc break-up for this sludge.

The activated sludge is subjected to shear forces during aeration. The mixing can result in both aggregation of dispersed cells and small flocs into larger segments and separation of larger flocs into smaller segments. Galil et al (1991) studied the influence of mixing, expressed in terms of the velocity gradient G, on the size of the sludge flocs. Four bench-scale activated sludge plants, operated with different agitation intensity (G-values: 64.1, 70.5, 107.1 and  $173.5 \text{ s}^{-1}$ ), were run in parallel. It was found that the size of the flocs were reciprocal functions of turbulence. Microscopic observation showed that the activated sludge flocs followed a bimodal floc size distribution ( $>90\%$  were larger than  $300 \mu\text{m}$  and the rest were small) and that the number of small flocs increased with turbulence. The value of SVI increased with turbulence. The average reactor effluent suspended solids concentration was lowest in the reactor maintained at a G of  $70.5 \text{ s}^{-1}$  (for this specific sludge). Flocs which are produced and continuously recycled through the aeration reactor have relatively high sensitivity to velocity gradients. It was further pointed out that the sludge flocs are exposed to agitation during almost as long a time as the sludge age and that they are reflocculated during the separation step. The reflocculated flocs are then recirculated to the aeration basin to be reexposed to agitation. This process takes place many times for each sludge floc.

Wahlberg (1992) made batch flocculation tests (on sludge taken from various full-scale plants) to measure activated sludge flocculation characteristics. The flocculation was described by six theoretically based parameters;  $k_A$ ,  $k_B$ ,  $n_0$ ,  $\alpha$ ,  $\beta$  and  $\lambda$ .

They were fitted in a differential equation to describe the change in the primary particle number concentration resulting from the disappearance of primary particles due to aggregation into larger flocs:

$$n_t = \frac{Gk_B}{k_A} + (n_0 - \frac{Gk_B}{k_A})\exp(-k_A X G t)$$

where  $n_t$  and  $n_0$  are the primary particle number concentration at time  $t$  and  $0$ ,  $G$  is the root-mean-square velocity gradient,  $X$  is the mixed liquor suspended solids concentration,  $k_A$  is the floc aggregation rate coefficient and  $k_B$  is the floc breakup rate coefficient. This equation can be written in a more general form:

$$n_t = \alpha + \beta \exp(-\lambda t)$$

It was found that the activated sludge aggregation by means of mechanical stirring was rapid (measured as the decrease in supernatant suspended solids concentration). For a batch reactor 99% was completed in 10 minutes and for a continuous-flow flocculator it was completed within 20 minutes. Moreover, the equilibrium supernatant solids concentration after flocculation was independent of the initial aggregation degree, but the flocculation time required decreased with initial degree of aggregation. The equilibrium supernatant solids concentration,  $\alpha$ , decreased with increasing cell surface polysaccharide (CSP) concentration. The floc breakup coefficient,  $k_B$ , decreased with increasing CSP to a threshold level after which it was constant with respect to CSP concentration.

Floc breakup has mainly been related to the turbulence in the aeration tank. Less attention has been paid to the effect of turbulence in the transport system. Das et al (1993) studied the floc breakup in 24 different activated sludge wastewater treatment plants. Activated sludge samples were taken at different locations in the wastewater plant with a Kemmerer sampler. The sludge settled in the sampler to avoid unnecessary handling of the sample. For diffused air plants, the supernatant suspended solids concentration varied with the  $G$ -value. Fine bubble aeration gave in general better supernatant clarity than a coarse bubble aeration due to lower  $G$ -values (the oxygen transfer is more efficient for fine bubbles than for coarse bubbles). No clear relationship between  $G$ -value and supernatant suspended solids concentration could be noticed for mechanically aerated plants. However, the closer to the aerator the sample was taken, the higher was the suspended solids concentration in the supernatant. It was also found that tapered aeration gave lower suspended solids concentration in the outlet of the aeration tank than the other aeration methods. The presence of elbows and free falls in the transport system caused floc break-up.

From this literature review it seems as though different types of activated sludge flocs respond in different ways to shear forces. This means that the potential for improving the clarification process by means of mechanical flocculation varies from plant to plant.

## 7.4. Dissolved oxygen concentration

Starkey and Karr (1984) studied the effect of low dissolved oxygen concentration (DO) on the effluent turbidity in a full-scale activated sludge plant. During a period (9 months) with low DO (< 1 mg/l) the effluent turned turbid. When the DO was increased to above 2 mg/l the effluent became clearer within a few days. It was hypothesized that two mechanisms could explain this phenomenon: inhibition of exocellular polymer production and inhibition of eucaryote population. For a further verification of this phenomenon, a bench-scale experiment was conducted (fed with synthetic wastewater). Low DO concentrations gave higher turbidities due to inhibition of exocellular polymers and reduction in the number of eucaryote microorganisms. The turbidity of the effluent from the test reactor began to increase approximately 10 hours after the DO concentration was reduced (from 5 to 0.4 mg/l). When the aeration was increased, the turbidity decreased almost immediately. To test the effect of colloidal loading on the turbidity, kaolin was added to the influent. The turbidity increased more when the DO concentration was held low. This could be explained by the reduction in adsorptive capacity due to inhibition of the extracellular polymer production. Another explanation could be inhibition of the eucaryote (protozoa and rotifers) population. They keep the effluent clear by consuming free bacteria. Higher background turbidities were obtained at higher F/M ratios. The effect of low DO was lower at low F/M ratios.

Knudson et al (1982) investigated the effect of mixed liquor DO concentrations on the maximum substrate utilization rates of activated sludge and to determine whether this would change the viability of the microorganisms. It was found that the maximum utilization rates based on the total volatile suspended solids may increase with increasing DO concentration over the range 1.5-15 mg/l if the flocs are greater than about 400  $\mu\text{m}$  in diameter. In the reactor with high DO concentration a slight trend of larger floc sizes with increased DO concentration was observed.

## 7.5. Effect of starvation

The composition of wastewater affect the metabolic activity of the microorganisms and also their surface structure. Wu (1978) found that activated sludge microorganisms grown in nitrogen and phosphorus restricted media possess exceptionally large capsules and produce a higher surface electric charge per unit of dry weight.

Horan and Shanmugan (1986) studied the effect of starvation and nutrient depletion on the settling properties of activated sludge. Starvation caused a rapid utilization of the internal storage polymer poly-3-hydroxy butyrate (PHB) as well as a decline in sludge respiration rate. At the same time the SSVI increased. The concentration of exocellular polymers did not change during the starvation and it was proposed that the loss of settling ability was caused by cell lysis. Filamentous microorganisms could also be seen.

Ericsson and Eriksson (1988) studied the effect of pre-precipitation on the settling properties of activated sludge. They found that extreme pre-precipitation lead to an increase in SVI and an increased growth of filamentous microorganisms.

Echeverría et al (1993) made a studie to evaluate the influence of preaeration and preprecipitation

on the settling properties of activated sludge. It was found that by adding a preaeration or preprecipitation stage the F/M ratio and the sludge age will be modified and the SVI changes. A high coagulant dosage can cause an excessive removal of organic material and phosphorus which can disturb the biological process, producing pin-point flocs and bulking sludge.

## **7.6. Effect of flocculants**

The settling properties of activated sludge changes when chemicals are added. The type as well as the dosing point is decisive for the settling properties. Boller (1993) compared the influence of adding Fe (II)-salts and Fe(III)-salts at different dosing points (simultaneous and pre-precipitation). Fe(II)-salts gave higher settling velocities than Fe(III)-salts and simultaneous precipitation gave higher settling velocities than pre-precipitation. This has a large impact on the limiting flux in the settlers (which also influence the sludge age that can be obtained in the system).

## 8. Discussion

This literature review shows that the settling process is very complex. Settling is a physical process, but the settling properties of the activated sludge are dependent on both physical and biological/chemical factors. A considerable amount of research has been done to improve the design and operation of secondary sedimentation tanks, but there are still problems with too high concentrations of suspended solids in the effluent. There is probably a threshold value which cannot be exceeded only by improving the operation of the secondary settler. Instead more effort should be put on what is happening in the aeration tank and how the wastewater composition affects the settling properties of activated sludge flocs. This is, however, very difficult because of the many interdependent factors which are involved. The composition of the wastewater, which is dependent on the industries and households connected to the wastewater system, is an important factor. The introduction of new, more 'environmentally friendly', household chemicals will probably change the composition of the wastewater and thus the structure and microflora of the activated sludge flocs. More research is needed within this field.

The problem of bulking sludge is also extremely complicated due to the considerable number of different types of filamentous microorganisms. A small number of filaments produce a clear effluent since the flocs sweep small flocs during settling. Problems begin when they grow in excessive numbers. Bulking and foaming sludge have been studied a great deal and these phenomena are well understood and effective control strategies have been developed. In this report, this problem is only treated in brief, since the main aim of this work was to study the settling properties of non-filamentous microorganisms.

More research should be carried out to study the properties of non-filamentous microorganisms since the mechanisms of floc formation and disintegration are not fully understood. One interesting area to study is the size distribution of flocs and which factors affect it. It could be an important and useful tool in studying what happens in the different treatment steps. To study the formation of extracellular polymers is also interesting but better methods need to be developed. That polyvalent cations have an important role in the bioflocculation process is quite clear from the results found in earlier works. The question is how this can be used in practice. Are the concentrations of polyvalent cations like  $\text{Ca}^{2+}$  high enough in the wastewater to produce optimal bioflocculation? It would be interesting to know if the settling properties are better in hard waters than in soft.

To achieve very low concentrations of suspended solids in the effluent, other methods than settling have to be used. Filtration is already used at some wastewater plants to polish the effluent. The drawback is that the filters have to be backwashed regularly. Flotation is an other interesting technique which could be used for polishing of effluents.

# References

- Allen T, du Pont de Nemours E. I & Co. (1985) Sedimentation techniques of particle size measurement, in: Particle size analysis, Lloyd P. J. (Eds.) John Wiley & Sons Ltd., 25-45.
- Andreadakis A. D. (1993) Physical and chemical properties of activated sludge floc, *Wat. Res.*, Vol. 27, No. 12, 1707-1714.
- Audic J. M., Fayoux Ch., Lesty Y. and Brisset P. (1994) Sludge retention times distribution in clarifier: a key point for population dynamic and nutrients removal control, *Wat. Sci. Tech.*, Vol. 29, No. 7, 57-60.
- Barbusiński K. and Kościelniak H. (1995) Influence of substrate loading intensity on floc size in activated sludge process, *Wat. Res.*, Vol. 29, No. 7, 1703-1710.
- Bisogni, Jr. J. J. and Lawrence A. W. (1971) Relationship between biological solids retention time and settling characteristics of activated sludge, *Wat. Res.*, Vol. 5, 753-763.
- Blackall L. L. (1994) Molecular identification of activated sludge foaming bacteria, *Wat. Sci. Tech.*, Vol. 7, No. 7.
- Boller M. (1993) Removal of organic matter by physico-chemical mechanisms in wastewater treatment plants, *Wat. Sci. Tech.*, Vol. 27, No. 11, 167-183.
- Brown M. J. and Lester J. N. (1980) Comparison of bacterial extracellular polymer extraction methods, *Appl. Env. Micr.*, Vol. 40, No. 2, 179-185.
- Bruus J. H., Nielsen P. H. and Keiding K. (1992) On the stability of activated sludge flocs with implication to dewatering, *Wat. Res.*, Vol. 26, No. 12, 1597-1604.
- Busch P.L. and Stumm W. (1968) Chemical interactions in the aggregation of bacteria; Bioflocculation in wastewater, *Envir.Sci. Tech.*, Vol. 2, 49-53.
- Camp T. R. (1936) A study of the rational design of settling tanks, *Sewage Works Journal*, Vol. 8, No. 5, 742-758.
- Cashion B. S. and Keinath T. M. (1983) Influence of three factors on clarification in the activated sludge process, *J. WPCF.*, Vol. 55, No. 11, 1331-1337.
- Chao A. C. and Keinath T.M. (1979) Influence of process loading intensity on sludge clarification and thickening characteristics, *Wat. Res.*, Vol. 13, 1213-1223.
- Chapman D. T. (1983) The influence of process variables on secondary clarification, *J. WPCF.*, Vol 55, 1425-1434.

- Chapman D.T. (1985) Final settler performance during transient loading, *JWPCF.*, Vol. 57, No. 3, 227-234.
- Chiesa S.C. and Irvine R.L. (1985) Growth and control of filamentous microbes in activated sludge- An integrated hypothesis, *Wat. Res.*, Vol. 19, No. 4, 471-479.
- Cho S. H., Colin F., Sardin M. and Prost C. (1993) Settling velocity model of activated sludge, *Wat. Res.*, Vol. 27, No. 7, 1237-1242.
- Christensen B. E. (1989) The role of extracellular polysaccharides in biofilms - a minireview, *J. Biotech.*, No. 10, 181-202.
- Chudoba P. and Pujol R. (1994) Kinetic selection of microorganisms by means of a selector- Twenty years of progress: history, practice and problems, *Wat. Sci. Tech.*, Vol. 29, No. 7.
- Chudoba J., Grau P. and Ottová V. (1973) Control of activated sludge filamentous bulking. II Selection of microorganisms by means of a selector, *Wat. Res.*, Vol. 7, 1389-1406.
- Chudoba J., Ottová V. and Madera V. (1973) Control of activated sludge filamentous bulking. I Effect of the hydraulic regime or degree of mixing in an aeration tank, *Wat. Res.*, Vol. 7, 1163-1182.
- Chudoba J., Blahá J. and Madera V. (1974) Control of activated sludge filamentous bulking. III Effect of sludge loading, *Wat. Res.*, Vol. 8, 231-237.
- Chudoba J. (1985) Control of activated sludge filamentous bulking. VI Formation of basic principles, *Wat. Res.*, Vol. 19, No. 8, 1017-1022.
- Coe H.S. and Clevenger G.H. (1916) Determining thickener unit areas, *Trans. AIME*, Vol. 55, No. 3.
- Daigger G. T. and Roper R. E. (1985) The relationship between SVI and activated sludge settling characteristics, *WPCF. J.*, Vol. 57, No. 8, 859-866.
- Daigger G. T. (1995) Development of refined clarifier operating diagrams using an updated settling characteristics database, *Wat. Env. Res.*, Vol. 67, No. 1, 95-100.
- Dammel E. E. and Schroeder E. D. (1991) Density of activated sludge solids, *Wat. Res.*, Vol. 25, No. 7, 841-846.
- Das D., Keinath T. M., Parker D. S. and Wahlberg E. J. (1993) Flocc breakup in activated sludge plants, *J. Env. Res.*, Vol. 65, No. 2, 138-145.
- Dick R. I. and Vesilind P. A. (1969) The sludge volume index- what is it?, *J. WPCF.*, Vol. 41, No. 7, 1285-1291.

Dick R. I. (1976) Folklore in the design of final settling tanks, *J. WPCF.*, Vol. 48, No. 4, 633-644.

Echeverría E., Seco A. and Ferrer J. (1992) Study of the factors affecting activated sludge settling in domestic wastewater treatment plants, *Wat. Sci. Tech.*, Vol. 25, No. 4/5, 273-279.

Echeverría E., Seco A. and Ferrer J. (1993) Control of activated sludge settleability using preaeration and preprecipitation. *Wat. Res.*, Vol. 27, No. 2, 293-296.

Eikelboom D.H. and van Buijsen H.J.J. (1981) Microscopic sludge investigation manual, TNO Research Institute for Environmental Hygiene, Delft, The Netherlands.

Eliosov B. and Argaman Y. (1995) Hydrolysis of particulate organics in activated sludge systems, *Wat. Res.*, Vol. 29, No. 1, 155-163.

Ericsson B. and Eriksson L. (1988) Activated sludge characteristics in a phosphorus depleted environment. *Wat. Res.* Vol. 22, No. 2, 151-162.

Eriksson L. and Alm B. (1991) Study of flocculation mechanisms by observing effects of a complexing agent on activated sludge properties, *Wat. Sci. Tech.*, Vol. 24, No. 7, 21-28.

Eriksson L., Steen I. and Tendaj M. (1992) Evaluation of sludge properties at an activated sludge plant, *Wat. Sci. Tech.*, Vol. 25, No. 6, 251-265.

Eriksson L. and Axberg C. (1981) Direct influence of wastewater pollutants on flocculation and sedimentation behaviour in biological wastewater treatment-I. Model system E. Coli B, *Wat. Res.*, Vol. 15, 421-431.

Eriksson L. and Härdin A-M. (1984) Settling properties of activated sludge related to floc structure, *Wat. Sci. Tech.*, vol. 16, 55-68.

Eriksson L. and Alm B. (1992) Model system studies of formation and properties of flocs obtained with cationic polyelectrolytes, in: Chemical water and wastewater treatment II, Klute R. and Hahn H. H. (Eds.), Berlin, Heidelberg, New York: Springer-Verlag, Proc. 5th Gothenburg Symposium, 26-30 Sep., 1992, Nizza.

Fitch B. (1979) Sedimentation of flocculent suspensions: state of the art, *Am.Inst.Chem.Engrs.J.*, Vol. 25, 913-930.

Forster C.F. (1992) Oils, fats and greases in wastewater treatment, *J.Chem.Technol.Biotechnol.*, Vol. 55, 402-404.

Forster C.F. (1985) Factors involved in the settlement of activated sludge I; nutrients and surface polymers, *Wat. Res.*, Vol. 19, No. 10, 1259-1264.

Forster C. F. (1985) Factors involved in the settlement of activated sludge II; the binding of

polyvalent metals, *Wat. Res.*, Vol. 19, No. 10, 1265-1271.

Forster C. F. (1968) The surface of activated sludge particles in relation to their settling characteristics, *Wat. Res.*, Vol. 2, 767-776.

Friedrich E., Friedrich H., Heinze W., Jobst K., Richter H.-J. and Hermel W. (1993) Progress in characterization of sludge particles, *Wat. Sci. Tech.*, Vol. 28, No. 1, 145-148.

Frølund B., Keiding K. and Nielsen P. H. (1994) A comparative study of biopolymers from a conventional and an advanced activated sludge treatment plant, *Wat. Sci. Tech.*, Vol. 29, No. 7, 137-141.

Gabb D.M.D., Still D.A., Ekama G.A., Jenkins D. and Marais G.v.R. (1991) The selector effect on filamentous bulking in long sludge age systems, *Wat. Sci. Tech.*, Vol. 23, 867-877.

Galil N., Stahl N., Novak J. T. and Rebhun M. (1991) The influence of mixing on the physical characteristics of biological flocs, *Research Journal WPCF.*, Vol. 63, No. 5, 768-772.

Ganczarczyk J.J. (1994) Microbial aggregates in wastewater treatment, IAWQ 17th Biennial International Conference, 24-29 July, Budapest, Hungary, *Wat. Sci. Tech.*, Vol. 30, No. 8, 87-95.

Ganczarczyk J. J., Zahid W. M. and Li D.-H. (1992) Physical stabilization and embedding of microbial aggregates for light microscopy studies, *Wat. Res.*, Vol. 26, No. 12, 1695-1699.

Goodwin J. A. S. and Forster C. F. (1985) A further examination into the composition of activated sludge surface in relation to their settlement characteristics, *Wat. Res.*, Vol. 19, No. 4, 527-533.

Gregory J. (1993) The role of colloid interactions in solid-liquid separation, *Wat. Sci. Tech.*, Vol. 27, No. 19, 1-17.

Hazen A. (1904) On sedimentation, *Trans. ASCE*, Vol. 53, No. 980, 45-88.

Henze M., Dupont R, Grau P. and De la Sota A. (1993) Rising sludge in secondary settlers due to denitrification, *Wat. Res.*, Vol. 27, No. 2, 231-236.

Horan N. J. and Shanmugan P. (1986) Effects of starvation and nutrient depletion on the settling properties of activated sludge, *Wat. Res.*, Vol. 20, No. 5, 661-666.

Horan N. J. and Eccles C. R. (1986) Purification and characterization of extracellular polysaccharide from activated sludges, *Wat. Res.*, Vol. 20, No. 11, 1427-1432.

Hultman B., Löwén M., Karlsson V., Li P.H. and Molina L. (1991) Prediction of activated sludge sedimentation based on sludge indices, *Wat. Sci. Tech.*, Vol. 24, No. 79, 33-42.

Jenkins D. (1992) Towards a comprehensive model of activated sludge bulking and foaming, *Wat. Sci. Tech.*, Vol. 25, No. 6, 215-230.

Jenkins D. (1991) Bulking control in activated sludge systems, Notes presented at Course on Operation of wastewater treatment plants, Perugia.

Jorand F., Guicherd P., Urbain V., Manem J. and Block J.C. (1994) Hydrophobicity of activated sludge flocs and laboratory grown bacteria, IAWQ 17th Biennial International Conference, 24-29 July, Budapest, Hungary, *Wat. Sci. Tech.*, Vol. 30, No. 11, 211-218.

Jorand F., Zartarian F., Thomas F., Block J. C., Bottero J. Y., Villemin G., Urbain V. and Manem J. (1995) Chemical and structural (2D) linkage between bacteria within activated sludge flocs, *Wat. Res.*, Vol. 29, No. 7, 1639-1647.

Kakii K., Hasumi M., Shirakashi T. and Kuriyama M. (1990) Involvement of  $Ca^{2+}$  in the flocculation of *Kluyvera cryocrescens* KA-103, *J. Ferm. Bioengng.*, Vol. 69, 224-227.

Kappeler J. and Gujer W. (1994) Influence of wastewater composition and operation conditions on activated sludge bulking and scumming, IAWQ 17th Biennial International Conference, 24-29 July, Budapest, Hungary, *Wat. Sci. Tech.*, Vol. 30, No. 11, 181-189.

Karr P. R. and Keinath T. M. (1978) Influence of particle size on sludge dewaterability, *J. WPCF.*, Vol. 50, 1911-1928.

Kavanaugh M.C., Tate M. C., Trussell A. R., Trussell R. R., and Treweek G. S. (1980) Use of particle size distribution measurement for selection and control of solid liquid separation processes. In: *Particulate in Water: Characterization, Fate, Effects and Removal*. Kavanaugh M.C. and Leckie J.D. (Eds). *Am. Chem. Soc.*, Washington D.C.

Knocke W. R. and Zentkovich T. L. (1986) Effects of mean cell residence time and particle size distribution on activated sludge vacuum dewatering characteristics, *J. WPCF.*, Vol. 58, No. 12, 1118-1123.

Knudson M. K., Williamson K. J. and Nelson P. O. (1982) Influence of dissolved oxygen on substrate utilization kinetics of activated sludge, *J. WPCF.*, Vol. 54, No. 1, 52-60.

Konièek Z. and Burdych J. (1988) Effect of activated sludge processes on secondary settling tank efficiencies, *Wat. Sci. Tech.*, Vol. 20, No. 4/5, 153-163.

Lau A.O., Strom P.F. and Jenkins D. (1984) Growth kinetics of *Sphaerotilus natans* and a flocc-former in pure and dual continuous culture. *JWPCF.*, Vol. 56, No. 1, 41-51.

Leentvaar J. and Rebhun M. (1983) Strength of ferric hydroxide flocs, *Wat. Res.*, Vol. 17, No. 8, 895-902.

- Levine A. D., Tchobanoglous G. and Asano T. (1985) Characterization of the size distribution of contaminants in wastewater: treatment and reuse implications, *J. WPCF*, Vol. 57, No. 7, 805-816.
- Li D-H. and Ganczarzyk J.J. (1988) Flow through activated sludge flocs, *Wat. Res.*, Vol. 22, No. 6, 789-792
- Li D-H. and Ganczarzyk J. J. (1987) Stroboscopic determination of settling velocity, size and porosity of activated sludge flocs, *Wat. Res.*, Vol. 21, No. 3, 257-262.
- Li D-H. and Ganczarzyk J. J. (1992) Advective transport in activated sludge flocs, *Wat. Env. Res.*, Vol. 64, No. 3,
- Li D-H. and Ganczarzyk J. J. (1991) Size distribution of activated sludge flocs, *Research Journal WPCF*. Vol. 63, No. 5, 806-814.
- Li D-H. and Ganczarzyk J. J. (1993) Factors affecting dispersion of activated sludge flocs, *Wat. Env. Res.* Vol. 65, No. 3, 258-263.
- Li D-H. and Ganczarzyk J.J. (1990) Structure of activated sludge flocs, *Biotech. Bioeng.*, Vol. 35, 57-65.
- Loosdrecht M. C. M. van, Lyklema J., Norde W., and Zehnder A. J. B. (1989) Bacterial adhesion: a physicochemical approach, *Microb. Ecol.*, 17, 1-15.
- Loosdrecht M. C. M. van, Lyklema J., Norde W., Schraa G, and Zehnder A. J. B. (1987) Electrical mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion, *Appl. Environ. Microbiol.*, Vol. 53, No. 8, 1898-1901.
- Lovett D. A., Kavanagh B. V. and Herbert L.S. (1983) Effect of sludge age and substrate composition on the settling and dewatering characteristics of activated sludge, *Wat. Res.*, Vol. 17, No. 11, 1511-1515.
- Lumley D. J. (1985) Settling of activated sludge; A study of limiting factors and dynamic response, Lic. thesis, Chalmers University of Technology, Göteborg.
- Lumley D. J. and Balmér P. (1990) Solids transport in rectangular secondary settlers. paper presented at IAWPRC-IWSA Joint Specialist Conference: Coagulation, Flocculation, Filtration, Sedimentation and Flotation, 23-26 April.
- Magara Y. and Nambu S. (1976) Biological and physical properties of an activated sludge on settling characteristics, *Wat. Res.*, Vol. 10, 71-77.
- McKinney R.E. (1953) Staining bacterial polysaccharides, *J. Bacteriol.*, Vol. 66, 453.
- McKinney R.E. (1956) Biological flocculation, In: Biological treatment of sewage and

industrial wastes, Vol. 1, 88.

Metcalf L. and Eddy H.P. (1979) Wastewater engineering, treatment, disposal, use, McGraw-Hill Publishing Company Ltd.

Morgan J. W., Forster C. F. and Evison L. (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges, *Wat. Res.*, Vol. 24, No. 6, 743-750.

Nielsen P.H., Raunkjaer K., Norsker N.H., Jensen N.Aa. and Hvitved-Jacobsen T. (1992) Transformation of wastewater in sewer systems- a review, *Wat. Sci. Tech.*, Vol. 25, 17-31.

Novak J.T. and Haugan B.-E. (1981) Polymer extraction from activated sludge, *J. WPCF.*, Vol. 53, No. 9, 1420-1424.

Novák L., Larrea L., Wanner J. and Garcia-Heras J. L. (1993) Non-filamentous activated sludge bulking in a laboratory scale system, *Wat. Res.*, Vol. 27, No. 8, 1339-1346.

Novák L., Larrea L., Wanner J. and García-Heras J. L. (1994) Non-filamentous activated sludge bulking caused by *Zoogloea*, *Wat. Sci. Tech.*, Vol. 29, No. 7, 301-304.

Parker D. S., Kaufman W. J. and Jenkins D. (1971) Physical conditioning of activated sludge, *J. WPCF.*, Vol. 43, No. 9, 1817-1833.

Parker D. S. (1983) Assessment of secondary clarification design concepts, *J. WPCF.*, Vol. 55, No. 4, 349-358.

Pavoni J. L., Tenney M. W. and Echelberger W. F., Jr. (1972) Bacterial exocellular polymers and biological flocculation, *J. WPCF.*, Vol. 44, No. 3, 414-431.

Pflanz P. (1969) Performance of activated sludge secondary sedimentation basins, IAWPR, Advances in Water Pollution Research, Proc. of 4th Intl. Conf. in Prague, S. H. Jenkins, Ed., Pergamon Press, Oxford, 569-593.

Pipes W. O. (1979) Bulking, deflocculation, and pinpoint floc, *J. WPCF.*, Vol. 51, No. 1, 62-70.

Pujol R. and Canler J.P. (1992) Biosorption and dynamics of bacterial populations in activated sludge, *Wat. Res.*, Vol. 26, No. 2, 209-212.

Rensink J.H. and Donker H.J.G.W. (1990) Control of bulking sludge in agro-industrial treatment plants, *Wat. Sci. Tech.*, Vol. 22, No. 9, 101-111.

Rensink J.H. (1966) Report pilot plant studies with dairy waste water during 1965-1966. Dairy Institute for Wastewater, Arnhem, The Netherlands, in Dutch.

- Rensink J.H. and Donker H.J.G.W. (1991) The effect of contact tank operation on bulking sludge and biosorption processes, *Wat. Sci. Tech.*, Vol. 23, 857-866.
- Rensink J.H. (1974) New approaches to preventing bulking sludge, *JWPCF.*, Vol. 46, 1888-1894.
- Sanin F. D. and Vesilind P. A. (1994) Effect of centrifugation on the removal of extracellular polymers and physical properties of activated sludge, IAWQ 17th Biennial International Conference, 24-29 July, Budapest, Hungary, *Wat. Sci. Tech.*, Vol. 30, No. 8, 117-127.
- Sezgin M., Jenkins D. and Parker D.S. (1978) A unified theory of filamentous sludge bulking, *JWPCF.*, Vol. 50, 362-381.
- Sheintuch M., Lev O., Einav P. and Rubin E. (1986) Role of exocellular polymer in the design of activated sludge, *Biotechn. Bioeng.*, Vol. 28, 1564-1576.
- Slijkhuis H. (1983) The Physiology of the filamentous bacterium *Microthrix parvicella*, PhD thesis, Agricultural College of Wageningen, The Netherlands.
- Starkey J. E. and Karr J. E. (1984) Effect of low dissolved oxygen concentration on effluent turbidity, *J. WPCF.*, Vol. 56, No. 7, 837-843.
- Steiner A. E., McLaren D. A. and Forster C. F. (1976) The nature of activated sludge flocs, *Wat. Res.*, Vol. 10, 25-30.
- Tambo N. and Hozumi H. (1979) Physical aspects of flocculation process-II. Contact flocculation, *Wat. Res.*, Vol. 13, No. 5, 441-448.
- Tambo N. and Watanabe Y. (1979) Physical aspect of flocculation process-I. Fundamental treatise, *Wat. Res.*, Vol. 13, No. 5, 429-439.
- Tambo N. and Watanabe Y. (1979) Physical characteristics of flocs I. The floc density function and aluminium floc, *Wat. Res.*, Vol. 13, No. 5, 409-419.
- Tambo N. and Hozumi H. (1979) Physical characteristics of flocs II. Strength of floc, *Wat. Res.*, Vol. 13, No. 5, 421-427.
- Tenney M. W. and Stumm W. (1965) Chemical flocculation of microorganisms in biological waste treatment, *J. WPCF.*, Vol. 37, No. 10, 1370-1388.
- Torrijos M., Cerro R.-M., Capdeville B., Zeghal S., Payraudeau M. and Lesouef A. (1994) Sequencing batch reactor: a tool for wastewater characterization for the IAWPRC model, *Wat. Sci. Tech.*, Vol. 29, No. 7, 81-90.
- Tuntoolavest M., Miller E. and Grady L. Jr. (1983) Factors affecting the clarification performance of activated sludge final settlers, *J. WPCF.*, Vol. 55, No. 3, 234-247.

Turakhia M.H., Cooksey K.E. and Characklis W.G. (1983) Influence of calcium-specific chelant on biofilm removal, *Appl. Environ. Microbiol.*, Vol. 46, 1236-1238.

Urbain V., Block J. C. and Manem J. (1993) Bioflocculation in activated sludge: an analytic approach, *Wat. Res.*, Vol. 27, No. 5, 829-838.

Wahlberg E. J. (1992) Activated sludge bioflocculation: measurement, influencing factors, and physical enhancement, PhD thesis, Clemson University, Clemson, USA.

Wanner J. (1994) The implementation of bulking control in the design of activated sludge systems, *Wat. Sci. Tech.*, Vol. 29, No. 7, 193-202.

Wanner J. (1992) Kinetic and metabolic selection in controlling the filamentous organisms in activated sludge. Notes presented at Course on Operation of Wastewater Treatment Plants, Perugia.

Wanner J. (1994) Activated sludge population dynamics, IAWQ 17th Biennial International Conference, 24-29 July, Budapest, Hungary, *Wat. Sci. Tech.*, Vol. 30, No. 11, 159-169.

Wanner J. and Grau P. (1988) Filamentous bulking in nutrient removal activated sludge systems, *Wat. Sci. Tech.*, Vol. 20, No. 4/5, 1-8.

Wu Y. (1978) Chemical flocculability of sludge organisms in response to growth conditions, *Biotechn. Bioeng.*, Vol. 20, 677-696.

Zahid W.M. and Ganczarczyk J. J. (1990) Suspended solids in biological filter effluents. *Wat. Res.*, Vol. 24, No. 2, 215-220.

Zita A. and Hermansson M. (1994) Effects of ionic strength on bacterial adhesion and stability of flocs in a wastewater activated sludge system, *Appl. Environ. Microbiol.*, Vol. 60, No. 9, 3041-3048.





